

FORM PTO-1390 (Modified) (REV 11-2000)		U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		ATTORNEY'S DOCKET NUMBER	
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371				RTSP-0153	
				U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR 09/937646	
INTERNATIONAL APPLICATION NO. PCT/US00/07634		INTERNATIONAL FILING DATE 23 March 2000		PRIORITY DATE CLAIMED 29 March 1999	
TITLE OF INVENTION Antisense Modulation of SRA Expression					
APPLICANT(S) FOR DO/EO/US COWSERT, Lex M. et al.					
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:					
<ol style="list-style-type: none"> 1. <input checked="" type="checkbox"/> This is a FIRST submission of items concerning a filing under 35 U.S.C. 371. 2. <input type="checkbox"/> This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371. 3. <input type="checkbox"/> This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (24) indicated below. 4. <input type="checkbox"/> The US has been elected by the expiration of 19 months from the priority date (Article 31). 5. <input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371 (c) (2)) <ol style="list-style-type: none"> a. <input type="checkbox"/> is attached hereto (required only if not communicated by the International Bureau). b. <input type="checkbox"/> has been communicated by the International Bureau. c. <input checked="" type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US). 6. <input type="checkbox"/> An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)). <ol style="list-style-type: none"> a. <input type="checkbox"/> is attached hereto. b. <input type="checkbox"/> has been previously submitted under 35 U.S.C. 154(d)(4). 7. <input checked="" type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3)) <ol style="list-style-type: none"> a. <input type="checkbox"/> are attached hereto (required only if not communicated by the International Bureau). b. <input type="checkbox"/> have been communicated by the International Bureau. c. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired. d. <input checked="" type="checkbox"/> have not been made and will not be made. 8. <input type="checkbox"/> An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)). 9. <input checked="" type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)). 10. <input type="checkbox"/> An English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)). 11. <input checked="" type="checkbox"/> A copy of the International Preliminary Examination Report (PCT/IPEA/409). 12. <input checked="" type="checkbox"/> A copy of the International Search Report (PCT/ISA/210). 					
<p>Items 13 to 20 below concern document(s) or information included:</p> <ol style="list-style-type: none"> 13. <input type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98. 14. <input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included. 15. <input checked="" type="checkbox"/> A FIRST preliminary amendment. 16. <input type="checkbox"/> A SECOND or SUBSEQUENT preliminary amendment. 17. <input type="checkbox"/> A substitute specification. 18. <input type="checkbox"/> A change of power of attorney and/or address letter. 19. <input checked="" type="checkbox"/> A computer-readable form of the sequence listing in accordance with 37 CFR 1.821-1.825. 20. <input type="checkbox"/> A second copy of the published international application under 35 U.S.C. 1.821-1.825. 21. <input type="checkbox"/> A second copy of the English language translation of the international application. 22. <input type="checkbox"/> Certificate of Mailing by Express Mail. 23. <input checked="" type="checkbox"/> Other items or information: 					
<p>1) Courtesy copy of International Application 2) Statement to Support Filing and Submission in Accordance with 37 CFR 1.821-1.825 and amended sequence list and diskette containing computer readable copy of Sequence Listing; 3) Return post card</p>					
				<p>"Express Mail" Label No. EL750774475US Date of Deposit September 27, 2001</p> <p>I hereby certify that this paper is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to the Assistant Commissioner for Patents, Box PCT, Washington, D.C. 20231.</p> <p>By <u>Deborah Ehret</u> Typed Name: Deborah Ehret</p>	

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR 1.492(a)(2)) 09/937646		INTERNATIONAL APPLICATION NO. PCT/US00/07634		ATTORNEY'S DOCKET NUMBER RTSP-0153	
---	--	--	--	--	--

24. The following fees are submitted:

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :				CALCULATIONS PTO USE ONLY	
<input type="checkbox"/>	Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO	\$1000.00			
<input checked="" type="checkbox"/>	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO	\$860.00			
<input type="checkbox"/>	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO	\$710.00			
<input type="checkbox"/>	International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4)	\$690.00			
<input type="checkbox"/>	International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4)	\$100.00			
ENTER APPROPRIATE BASIC FEE AMOUNT =			\$860.00		
Surcharge of \$130.00 for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).				\$0.00	
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	17 - 20 =	0	x \$18.00	\$0.00	
Independent claims	1 - 3 =	0	x \$80.00	\$0.00	
Multiple Dependent Claims (check if applicable).			<input type="checkbox"/>	\$0.00	
TOTAL OF ABOVE CALCULATIONS =				\$860.00	
<input checked="" type="checkbox"/> Applicant claims small entity status. (See 37 CFR 1.27). The fees indicated above are reduced by 1/2.				\$430.00	
SUBTOTAL =				\$430.00	
Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492 (f)).				\$0.00	
TOTAL NATIONAL FEE =				\$430.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) (check if applicable).				\$0.00	
TOTAL FEES ENCLOSED =				\$430.00	
Applicant, ISIS Pharmaceuticals, Inc. is entitled to small entity status. It is a small business concern.				Amount to be: refunded	\$
				charged	\$

a. ☒ A check in the amount of **\$430.00** to cover the above fees is enclosed.

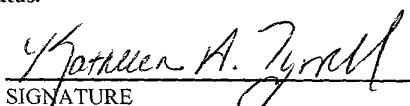
b. ☐ Please charge my Deposit Account No. _____ in the amount of _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **50-1619**. A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Jane Massey Licata, Reg. No. 32,257 Kathleen A. Tyrrell, Reg. No. 38,350 Licata & Tyrrell P.C. 66 E. Main Street Marlton, New Jersey 08053 Telephone: (856) 810-1515 Facsimile: (856) 810-1454	 SIGNATURE Kathleen A. Tyrrell NAME 38,350 REGISTRATION NUMBER September 27, 2001 DATE
---	--

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Attorney Docket No.: RTSP-0153
Inventors: Cowsert et al.
Serial No.: Not yet assigned
Filing Date: Not yet assigned
Examiner: Not yet assigned
Group Art Unit: Not yet assigned
Title: Antisense Modulation of SRA Expression

"Express Mail" Label No. EL 750774475 US
Date of Deposit - September 27, 2001

I hereby certify that this paper is being deposited with
the United States Postal Service "Express Mail Post Office
to Addressee" service under 37 CFR 1.10 on the date
indicated above and is addressed to the Assistant Commissioner for
Patents, Washington, D.C. 20231.

By Deborah Ehret
Typed Name: Deborah Ehret

BOX SEQUENCE
Assistant Commissioner for Patents
Washington, DC 20231

Sir:

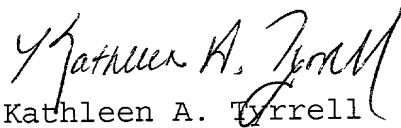
PRELIMINARY AMENDMENT

It is respectfully requested that the Sequence Listing of the
instant specification be deleted and replaced with the amended
Sequence Listing provided herewith. A paper copy and a CRF copy of
the amended Sequence Listing are provided herewith.

The replacement Sequence Listing has been amended to conform
with the current Sequence Listing Rules. However, data presented
in the Sequence Listing is identical to that originally filed.

Thus, no new matter has been added by this amendment.

Respectfully submitted,



Kathleen A. Tyrrell
Registration No. 38,350

Date: September 27, 2001

LICATA & TYRRELL P.C.
66 E. Main Street
Marlton, New Jersey 08053

(856) 810-1515

SEQUENCE LISTING

<110> Lex M. Cowser
C. Frank Bennett
Bert W. O'Malley

<120> ANTISENSE MODULATION OF SRA EXPRESSION

<130> RTSP-0153

<140>

<141>

<150> US 09/280,409

<151> 1999-03-29

<160> 146

<210> 1

<211> 626

<212> DNA

<213> Homo sapiens

<400> 1

```
ggcggaacca ggcgctcgct gcttaccaag agggtagccg caccacagga tggatcccc 60
agagtcctcg catcagagac ttctcctggg cctccccaa tggggcctcc acctccttca 120
agtaaggctc ccaggtcccc acctgtgggg agtggtcctg cctctggcgt ggagcccaca 180
agtttcccag tcgagtctga ggctcgactg atggaggatg tgctgagacc tttggaacag 240
gcattggaag actgccgtgg ccacacaagg aagcaggtat gtgatgacat cagccgacgc 300
ctggcactgc tgcaggaaca gtgggctgga ggaaagttgt caatacctgt aaagaagaga 360
atggctctac tgggtgcaaga gctttcaagc caccgggtggg acgcagcaga tgacatccac 420
cgctccctca tgggtgacca tgtgactgag gtcagtcagt ggatggtagg agttaaaga 480
ttaattgcag aaaagaggag tctgttttca gaggaggcag ccaatgaaga gaaatctgca 540
gccacagctg agaagaacca taccatacca ggcttccagc aggcttcata atcctcggtt 600
ccccagactc accggacacc atctcc 626
```

<210> 2

<211> 859

<212> DNA

<213> Homo sapiens

<400> 2

```
cggaacaag gaacgcggct ggaacgaccc gccgcagttc tcatacgggc tgcagacca 60
ggcggcgga cccaggcgtc cgctgcttac caagagggtc gccgcacccc aggatggatc 120
ccccagagtc cccgcatcag agacttctcc tgggcctccc ccaatggggc ctccacctcc 180
ttcaagtaag gctcccaggt cccacactgt ggggagtggt cctgcctctg gcgtggagcc 240
cacaagtttc ccagtcgagt ctgaggctcg actgatggag gatgtgctga gacctttgga 300
```

```

acaggcattg gaagactgcc gtggccacac aaggaagcag gtatgtgatg acatcagccg 360
acgcctggca ctgctgcagg aacagtgggc tggaggaaag ttgtcaatac ctgtaaagaa 420
gagaatggct ctactggtgc aagagctttc aagccaccgg tgggacgcag cagatgacat 480
ccaccgctcc ctcatggttg accatgtgac tgaggtcagt cagtggatgg taggagttaa 540
aagattaatt gcagaaaaga ggagtctgtt ttcagaggag gcagccaatg aagagaaatc 600
tgcagccaca gctgagaaga accataccat accaggcttc cagcaggctt cataatcctc 660
ggttccccag actcaccgga caccatctcc tatgccttgg agaccttctg tcaacttggct 720
cccttcttac caccaccaag actgtccac tgggcctgac ccacctatga gggaagaagt 780
cccacctggg ccagaggagg ttcatgtgtt actcataaca tgcatttcaa taaaaacatc 840
tctgcggtga aaaaaaaaaa 859

```

```

<210> 3
<211> 1086
<212> DNA
<213> Homo sapiens

```

```

<400> 3
gtagcgaccg gcgctcagct ggcgtcgccc cggcctaggc tgggggcggt tgcggcgctt 60
agtatggacc ctctgtctcc cccagcccca gtatcagcta acagtggagt tccgggcctc 120
gcttcacaca tccctcgcct ccgcaggcaa caaggaaagc ggctggaacg acccgccgca 180
gttctcatac gggctgcaga cccaggccgg cggaccaggc cgctcgctgc ttaccaagag 240
ggtagccgca cccaggatg gatccccag agtccccgca tcagagactt ctccctgggcc 300
tccccaatg gggcctccac ctccctcaag taaggctccc aggtccccac ctgtggggag 360
tggtcctgcc tctggcgtgg agcccacaag tttcccagtc gagtctgagg ctcgactgat 420
ggaggatgtg ctgagacctt tggaacaggc attggaagac tgccgtggcc acacaaggaa 480
gcaggatatg gatgacatca gccgacgcct ggcactgctg caggaacagt gggctggagg 540
aaagtgtgca atacctgtaa agaagagaat ggctctactg gtgcaagagc tttcaagcca 600
ccggtgggac gcagcagatg acatccaccg ctccctcatg gttgaccatg tgactgaggt 660
cagtcagtgg atggtaggag taaaagatt aattgcagaa aagaggagtc tgttttcaga 720
ggaggcagcc aatgaagaga aatctgcagc cacagctgag aagaaccata ccataccagg 780
cttcacagcag gcttcataat cctcggttcc ccagactcac cggacaccat ctccctccgg 840
tgccaageta gtccctctgg tgcctcgac tgccctgctc cctgtgtatc tgcaaacctc 900
tgtttccct tctccattca tcaggaaggg atctgctggg taaagtcaga ctactgccta 960
ccactttttc ccaaagtaga ctgaacaagc atcctgtgct gggcgagca gctgtgtttg 1020
gatggtttca tttcagcatg agaacagact caaatagaac gggccggaat tccgccgata 1080

```

20160324

ctgacg

1086

<210> 4
<211> 1123
<212> DNA
<213> Homo sapiens

<400> 4
actcctggag cccgtcagta tcggcggccc tcatcacgtc cccacactgg ctgtacagga 60
tcttgccatc cccactcgg tcaaacagct cgaaggcctc cttgaactcc tccagctggt 120
ccttggtaaa ctcgatcacc actttggaga gatcgactgg aggctcctgg gttttctgag 180
gggcctgggg gacagctggc tcagctttgg tcttggtctg aggagcccct gctgctgctg 240
gcttagcagc aggtttggag atggaggagc tgtacgtgaa gccgggcaac aaggaacgcg 300
gctggaacga cccgccgag ttctcatcag ggctgcagac ccaggccggc ggacccaggc 360
gctcgtgctg taccaagagg gtacgcgcac cccaggatgg atccccaga gtccccgcat 420
cagagacttc tcctgggcct cccccaatgg ggctccacc tccttcaagt aaggctccca 480
ggtccccacc tgtggggagt ggtcctgctc ctggcgtgga gccacaagt ttcccagtcg 540
agtctgaggg tcgactgatg gaggatgtgc tgagaccttt ggaacaggca ttggaagact 600
gccgtggcca cacaaggaag caggtatgtg atgacatcag ccgacgcctg gcaactgctgc 660
aggaacagtg ggctggagga aagttgtcaa tacctgtaaa gaagagaatg gctctactgg 720
tgcaagagct ttcaagccac cgggtgggacg cagcagatga catccaccgc tccctcatgg 780
ttgaccatgt gactgaggtc agtcagtgga tggtaggagt taaaagatta attgcagaaa 840
agaggagtct gtttttcagag gaggcagcca atgaagagaa atctgcagcc acagctgaga 900
agaaccatac cataccaggc ttccagcagg cttcataatc ctcggttccc cagactcacc 960
ggacaccatc tcctcaaata gaacggggag acttttccct caacaaaagg aaagacagtc 1020
ctatgtgcac tgtatcacc ttgagatact actgttacag agattagaac cacattgagt 1080
ggggttttct gtgtaaatcg aaggagaaaa agaccagatt act 1123

<210> 5
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR Primer

<400> 5
gtggccacac aaggaagca 19

<210> 6

<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR Primer

<400> 6
ctttcctcca gccactgtt c

21

<210> 7
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR Probe

<400> 7
atgacatcag ccgacgcctg ga

22

<210> 8
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR Primer

<400> 8
gaaggtgaag gtcggagtc

19

<210> 9
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR Primer

<400> 9
gaagatggtg atgggatttc

20

<210> 10
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR Probe

<400> 10
caagcttccc gttctcagcc

20

<210> 11
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Antisense Oligonucleotide

<400> 11 18
cgagcgctg ggtccgcc

<210> 12
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Antisense Oligonucleotide

<400> 12 18
accctcttgg taagcagc

<210> 13
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Antisense Oligonucleotide

<400> 13 18
gaggcccagg agaagtct

<210> 14
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Antisense Oligonucleotide

<400> 14 18
ggagccttac ttgaagga

<210> 15
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Antisense Oligonucleotide

<400> 15 18
accactcccc acaggtgg

<210> 16
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Antisense Oligonucleotide

<400>	16	
agaggcagga ccactccc		18
<210>	17	
<211>	18	
<212>	DNA	
<213>	Artificial Sequence	
<220>		
<223>	Antisense Oligonucleotide	
<400>	17	
gctccacgcc agaggcag		18
<210>	18	
<211>	18	
<212>	DNA	
<213>	Artificial Sequence	
<220>		
<223>	Antisense Oligonucleotide	
<400>	18	
aaacttgtgg gctccacg		18
<210>	19	
<211>	18	
<212>	DNA	
<213>	Artificial Sequence	
<220>		
<223>	Antisense Oligonucleotide	
<400>	19	
ctcgactggg aaacttgt		18
<210>	20	
<211>	18	
<212>	DNA	
<213>	Artificial Sequence	
<220>		
<223>	Antisense Oligonucleotide	
<400>	20	
gagcctcaga ctcgactg		18
<210>	21	
<211>	18	
<212>	DNA	
<213>	Artificial Sequence	
<220>		
<223>	Antisense Oligonucleotide	
<400>	21	
ctccatcagt cgagcctc		18

<210>	22	
<211>	18	
<212>	DNA	
<213>	Artificial Sequence	
<220>		
<223>	Antisense Oligonucleotide	
<400>	22	
	cagcacatcc tccatcag	18
<210>	23	
<211>	18	
<212>	DNA	
<213>	Artificial Sequence	
<220>		
<223>	Antisense Oligonucleotide	
<400>	23	
	ttccaaaggt ctcagcac	18
<210>	24	
<211>	18	
<212>	DNA	
<213>	Artificial Sequence	
<220>		
<223>	Antisense Oligonucleotide	
<400>	24	
	caatgcctgt tccaaagg	18
<210>	25	
<211>	18	
<212>	DNA	
<213>	Artificial Sequence	
<220>		
<223>	Antisense Oligonucleotide	
<400>	25	
	cggcagtctt ccaatgcc	18
<210>	26	
<211>	18	
<212>	DNA	
<213>	Artificial Sequence	
<220>		
<223>	Antisense Oligonucleotide	
<400>	26	
	tgtgtggcca cggcagtc	18
<210>	27	

<211> 18
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Antisense Oligonucleotide

 <400> 27
 acctgcttcc ttgtgtgg 18

 <210> 28
 <211> 18
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Antisense Oligonucleotide

 <400> 28
 tgatcatcaca tacctgct 18

 <210> 29
 <211> 18
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Antisense Oligonucleotide

 <400> 29
 cgtcggctga tgatcatca 18

 <210> 30
 <211> 18
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Antisense Oligonucleotide

 <400> 30
 cagtgcagg cgtcggct 18

 <210> 31
 <211> 18
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Antisense Oligonucleotide

 <400> 31
 tggtcctgca gcagtgc 18

 <210> 32
 <211> 18
 <212> DNA
 <213> Artificial Sequence

<220>			
<223>	Antisense Oligonucleotide		
<400>	32		18
	ctccagccca ctgttcct		
<210>	33		
<211>	18		
<212>	DNA		
<213>	Artificial Sequence		
<220>			
<223>	Antisense Oligonucleotide		
<400>	33		18
	tgacaacttt cctccagc		
<210>	34		
<211>	18		
<212>	DNA		
<213>	Artificial Sequence		
<220>			
<223>	Antisense Oligonucleotide		
<400>	34		18
	tttacaggta ttgacaac		
<210>	35		
<211>	18		
<212>	DNA		
<213>	Artificial Sequence		
<220>			
<223>	Antisense Oligonucleotide		
<400>	35		18
	ccattctctt ctttacag		
<210>	36		
<211>	18		
<212>	DNA		
<213>	Artificial Sequence		
<220>			
<223>	Antisense Oligonucleotide		
<400>	36		18
	gcaccagtag agccattc		
<210>	37		
<211>	18		
<212>	DNA		
<213>	Artificial Sequence		
<220>			
<223>	Antisense Oligonucleotide		

<400>	37	
tgaaagctct tgcaccag		18
<210>	38	
<211>	18	
<212>	DNA	
<213>	Artificial Sequence	
<220>		
<223>	Antisense Oligonucleotide	
<400>	38	
ccaccggtgg cttgaaag		18
<210>	39	
<211>	18	
<212>	DNA	
<213>	Artificial Sequence	
<220>		
<223>	Antisense Oligonucleotide	
<400>	39	
atctgctgcg tcccaccg		18
<210>	40	
<211>	18	
<212>	DNA	
<213>	Artificial Sequence	
<220>		
<223>	Antisense Oligonucleotide	
<400>	40	
gtggatgtca tctgctgc		18
<210>	41	
<211>	18	
<212>	DNA	
<213>	Artificial Sequence	
<220>		
<223>	Antisense Oligonucleotide	
<400>	41	
atgaggagc ggtggatg		18
<210>	42	
<211>	18	
<212>	DNA	
<213>	Artificial Sequence	
<220>		
<223>	Antisense Oligonucleotide	
<400>	42	
catggtcaac catgaggg		18

<210>	43	
<211>	18	
<212>	DNA	
<213>	Artificial Sequence	
<220>		
<223>	Antisense Oligonucleotide	
<400>	43	18
	ctgacctcag tcacatgg	
<210>	44	
<211>	18	
<212>	DNA	
<213>	Artificial Sequence	
<220>		
<223>	Antisense Oligonucleotide	
<400>	44	18
	accatccact gactgacc	
<210>	45	
<211>	18	
<212>	DNA	
<213>	Artificial Sequence	
<220>		
<223>	Antisense Oligonucleotide	
<400>	45	18
	ctttttaactc ctaccatc	
<210>	46	
<211>	18	
<212>	DNA	
<213>	Artificial Sequence	
<220>		
<223>	Antisense Oligonucleotide	
<400>	46	18
	cttttctgca attaatct	
<210>	47	
<211>	18	
<212>	DNA	
<213>	Artificial Sequence	
<220>		
<223>	Antisense Oligonucleotide	
<400>	47	18
	aaaacagact cctctttt	
<210>	48	

<211> 18
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Antisense Oligonucleotide

 <400> 48
 ttggctgcct cctctgaa 18

 <210> 49
 <211> 18
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Antisense Oligonucleotide

 <400> 49
 gggtcttctc agctgtgg 18

 <210> 50
 <211> 18
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Antisense Oligonucleotide

 <400> 50
 ggaaccgagg attatgaa 18

 <210> 51
 <211> 18
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Antisense Oligonucleotide

 <400> 51
 ccgcgttcct tgttgccg 18

 <210> 52
 <211> 18
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Antisense Oligonucleotide

 <400> 52
 ttccagccgc gttccttg 18

 <210> 53
 <211> 18
 <212> DNA
 <213> Artificial Sequence

<220>		
<223>	Antisense Oligonucleotide	
<400>	53	
aactgcggcg	ggtcgttc	18
<210>	54	
<211>	18	
<212>	DNA	
<213>	Artificial Sequence	
<220>		
<223>	Antisense Oligonucleotide	
<400>	54	
ggcctgggtc	tcgagccc	18
<210>	55	
<211>	18	
<212>	DNA	
<213>	Artificial Sequence	
<220>		
<223>	Antisense Oligonucleotide	
<400>	55	
cctgggtccg	ccggcctg	18
<210>	56	
<211>	18	
<212>	DNA	
<213>	Artificial Sequence	
<220>		
<223>	Antisense Oligonucleotide	
<400>	56	
tggtgaagcag	cgagcgcc	18
<210>	57	
<211>	18	
<212>	DNA	
<213>	Artificial Sequence	
<220>		
<223>	Antisense Oligonucleotide	
<400>	57	
ggcccaggag	aagtctct	18
<210>	58	
<211>	18	
<212>	DNA	
<213>	Artificial Sequence	
<220>		
<223>	Antisense Oligonucleotide	

<400>	58	
tgaaggaggt	ggaggccc	18
<210>	59	
<211>	18	
<212>	DNA	
<213>	Artificial Sequence	
<220>		
<223>	Antisense Oligonucleotide	
<400>	59	
gaccactccc	cacaggtg	18
<210>	60	
<211>	18	
<212>	DNA	
<213>	Artificial Sequence	
<220>		
<223>	Antisense Oligonucleotide	
<400>	60	
actgggaaac	ttgtgggc	18
<210>	61	
<211>	18	
<212>	DNA	
<213>	Artificial Sequence	
<220>		
<223>	Antisense Oligonucleotide	
<400>	61	
tcagcacatc	ctccatca	18
<210>	62	
<211>	18	
<212>	DNA	
<213>	Artificial Sequence	
<220>		
<223>	Antisense Oligonucleotide	
<400>	62	
tggccacggc	agtcttcc	18
<210>	63	
<211>	18	
<212>	DNA	
<213>	Artificial Sequence	
<220>		
<223>	Antisense Oligonucleotide	
<400>	63	
cctgcttcct	tgtgtggc	18

<210>	64	
<211>	18	
<212>	DNA	
<213>	Artificial Sequence	
<220>		
<223>	Antisense Oligonucleotide	
<400>	64	
	gtcggctgat gtcacac	18
<210>	65	
<211>	18	
<212>	DNA	
<213>	Artificial Sequence	
<220>		
<223>	Antisense Oligonucleotide	
<400>	65	
	ctttcctcca gccactg	18
<210>	66	
<211>	18	
<212>	DNA	
<213>	Artificial Sequence	
<220>		
<223>	Antisense Oligonucleotide	
<400>	66	
	ctgctgcgtc ccaccgt	18
<210>	67	
<211>	18	
<212>	DNA	
<213>	Artificial Sequence	
<220>		
<223>	Antisense Oligonucleotide	
<400>	67	
	agcggtgat gtcacatg	18
<210>	68	
<211>	18	
<212>	DNA	
<213>	Artificial Sequence	
<220>		
<223>	Antisense Oligonucleotide	
<400>	68	
	tcacatggc aaccatga	18
<210>	69	

<211> 18
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Antisense Oligonucleotide

 <400> 69
 ttttaactcc taccatcc 18

 <210> 70
 <211> 18
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Antisense Oligonucleotide

 <400> 70
 tttctgcaat taatcttt 18

 <210> 71
 <211> 18
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Antisense Oligonucleotide

 <400> 71
 cctctgaaaa cagactcc 18

 <210> 72
 <211> 18
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Antisense Oligonucleotide

 <400> 72
 tttctcttca ttggctgc 18

 <210> 73
 <211> 18
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Antisense Oligonucleotide

 <400> 73
 ctcagctgtg gctgcaga 18

 <210> 74
 <211> 18
 <212> DNA
 <213> Artificial Sequence

PTO/PCT Rec'd 27 SEP 2001

ANTISENSE MODULATION OF SRA EXPRESSION

FIELD OF THE INVENTION

The present invention provides compositions and methods for modulating the expression of SRA. In particular, this invention relates to antisense compounds, particularly oligonucleotides, specifically hybridizable with SRA or nucleic acids encoding human SRA. Such oligonucleotides have been shown to modulate the expression of SRA.

10 BACKGROUND OF THE INVENTION

Steroid, thyroid and retinoid hormones produce a diverse array of physiologic effects through the regulation of gene expression. Upon entering the cell, these hormones bind to a unique group of intracellular nuclear receptors which have been characterized as ligand-dependent transcription factors. This complex then moves into the nucleus where the receptor and its cognate ligand interact with the transcription preinitiation complex affecting its stability and ultimately the rate of transcription of the target genes.

The interactions of the liganded receptor with the specific elements in the promoter region of the target genes are mediated by multi-protein complexes. These complexes are composed of either corepressors, which inhibit transactivation or coactivators, which enhance transactivation (Glass et al., *Curr. Opin. Cell. Biol.*, 1997, 9, 222-232). Several nuclear receptor coactivator proteins have been identified, all having similar biochemical properties. Recently, however, a novel coactivator was reported to be the first endogenous RNA transcript to function as an eukaryotic transcriptional coactivator. This coactivator, SRA, or steroid receptor RNA activator, has several unique features. SRA selectively interacts with the AF-1 domain of steroid

-2-

hormone receptors found in the amino terminus of the receptor. Nuclear receptors contain two AF (activation function) domains responsible for their transcription activation function, and previously identified

5 coactivators have all been shown to interact through the AF-2 domain.

SRA is also expressed as multiple isoforms in a tissue and cell-specific manner. Multiple transcripts of SRA were isolated and fell into two categories; a major transcript
10 fraction of 0.7-0.85 kilobases (kb) in length producing a doublet and a minor transcript fraction of 1.3-1.5 kilobases. Of the two major transcripts in the doublet, the 0.7 kb species was expressed at significantly higher levels in breast cancer cell lines. All of the transcripts were
15 found to exist at different levels in the tissues examined but were enriched in the liver and skeletal muscle.

Finally, SRA was found to be present in a preformed coregulator complex with the AF-2 coactivator, SRC-1. SRC-1 or steroid receptor coactivator-1 has been shown to
20 enhance the transcriptional activity of the estrogen, glucocorticoid, thyroid, progesterone and retinoic acid receptors (Onate et al., *Science*, 1995, 270, 1354-1357). In immunoprecipitation experiments, it was shown that SRA acts as a component of distinct ribonucleoprotein
25 complexes, one of which contains the nuclear receptor coactivator, SRC-1. Thus the SRA mRNA transcript itself, specifically expressed in steroid target tissues, functions as a component of a large multiprotein complex to selectively enhance transcriptional activation by steroid
30 receptors.

Currently, there are no known therapeutic agents that effectively inhibit the synthesis of SRA. Consequently, there remains a long felt need for agents capable of selectively and effectively inhibiting SRA function.

-3-

Antisense technology is emerging as an effective means for reducing the expression of specific gene products and may therefore prove to be uniquely useful in a number of therapeutic, diagnostic, and research applications for the modulation of SRA expression.

SUMMARY OF THE INVENTION

The present invention is directed to antisense compounds, particularly oligonucleotides, which are targeted to SRA or a nucleic acid encoding SRA, and which modulate SRA or the expression of SRA. Pharmaceutical and other compositions comprising the antisense compounds of the invention are also provided. Further provided are methods of modulating levels of SRA in cells or tissues comprising contacting said cells or tissues with one or more of the antisense compounds or compositions of the invention. Further provided are methods of treating an animal, particularly a human, suspected of having or being prone to a disease or condition associated with SRA by administering a therapeutically or prophylactically effective amount of one or more of the antisense compounds or compositions of the invention.

DETAILED DESCRIPTION OF THE INVENTION

The present invention employs oligomeric antisense compounds, particularly oligonucleotides, for use in modulating the function of SRA or nucleic acid molecules encoding SRA, ultimately modulating the amount of SRA present. This is accomplished by providing antisense compounds which specifically hybridize with an SRA transcript or nucleic acids encoding SRA. As used herein, the terms "target nucleic acid" and "nucleic acid encoding SRA" encompass SRA, DNA encoding SRA, RNA (including pre-mRNA and mRNA) transcribed from such DNA, and also cDNA derived from such RNA. The specific hybridization of an oligomeric compound with its target nucleic acid interferes with the normal function of the nucleic acid. This

-4-

modulation of function of a target nucleic acid by compounds which specifically hybridize to it is generally referred to as "antisense". The functions of DNA to be interfered with include replication and transcription. The functions of RNA to be interfered with include all vital functions such as, for example, translocation of the RNA to the site of protein translation, translation of protein from the RNA, splicing of the RNA to yield one or more mRNA species, and catalytic activity which may be engaged in or facilitated by the RNA. The overall effect of such interference with target nucleic acid function is modulation of the expression of SRA. In the context of the present invention, "modulation" means either an increase (stimulation) or a decrease (inhibition) in the expression of a gene. In the context of the present invention, inhibition is the preferred form of modulation of gene expression and mRNA is a preferred target. Because it is presently believed that SRA may not be translated and the SRA mRNA transcript itself may be the functional moiety in transcriptional coactivation, the target molecule is the SRA mRNA transcript itself, which may then be inactivated or degraded through antisense activity, or a nucleic acid encoding SRA, antisense targeting to which would then block SRA expression.

It is preferred to target specific nucleic acids for antisense. "Targeting" an antisense compound to a particular nucleic acid, in the context of this invention, is a multistep process. The process usually begins with the identification of a nucleic acid sequence whose function is to be modulated. This may be, for example, a cellular gene (or mRNA transcribed from the gene) whose expression is associated with a particular disorder or disease state, or a nucleic acid molecule from an infectious agent. In the present invention, the target is SRA or a nucleic acid molecule encoding SRA. The targeting

-5-

process also includes determination of a site or sites within this gene for the antisense interaction to occur such that the desired effect, e.g., detection or modulation of expression of the protein, will result. Within the

5 context of the present invention, a preferred intragenic site is the region encompassing the translation initiation or termination codon of the open reading frame (ORF) of the gene. Since, as is known in the art, the translation initiation codon is typically 5'-AUG (in transcribed mRNA

10 molecules; 5'-ATG in the corresponding DNA molecule), the translation initiation codon is also referred to as the "AUG codon," the "start codon" or the "AUG start codon". A minority of genes have a translation initiation codon having the RNA sequence 5'-GUG, 5'-UUG or 5'-CUG, and

15 5'-AUA, 5'-ACG and 5'-CUG have been shown to function in vivo. Thus, the terms "translation initiation codon" and "start codon" can encompass many codon sequences, even though the initiator amino acid in each instance is typically methionine (in eukaryotes) or formylmethionine

20 (in prokaryotes). It is also known in the art that eukaryotic and prokaryotic genes may have two or more alternative start codons, any one of which may be preferentially utilized for translation initiation in a particular cell type or tissue, or under a particular set

25 of conditions. In the context of the invention, "start codon" and "translation initiation codon" refer to the codon or codons, if any, that would be used in vivo to initiate translation of an mRNA molecule transcribed from a gene encoding SRA, regardless of the sequence(s) of such

30 codons.

It is also known in the art that a translation termination codon (or "stop codon") of a gene may have one of three sequences, i.e., 5'-UAA, 5'-UAG and 5'-UGA (the corresponding DNA sequences are 5'-TAA, 5'-TAG and 5'-TGA,

35 respectively). The terms "start codon region" and

-6-

"translation initiation codon region" refer to a portion of such an mRNA or gene that encompasses from about 25 to about 50 contiguous nucleotides in either direction (i.e., 5' or 3') from a translation initiation codon. Similarly, the terms "stop codon region" and "translation termination codon region" refer to a portion of such an mRNA or gene that encompasses from about 25 to about 50 contiguous nucleotides in either direction (i.e., 5' or 3') from a translation termination codon.

10 The open reading frame (ORF) or "coding region," which is known in the art to refer to the region between the translation initiation codon and the translation termination codon, is also a region which may be targeted effectively. Other target regions include the 5' untranslated region (5'UTR), known in the art to refer to the portion of an mRNA in the 5' direction from the translation initiation codon, and thus including nucleotides between the 5' cap site and the translation initiation codon of an mRNA or corresponding nucleotides on the gene, and the 3' untranslated region (3'UTR), known in the art to refer to the portion of an mRNA in the 3' direction from the translation termination codon, and thus including nucleotides between the translation termination codon and 3' end of an mRNA or corresponding nucleotides on the gene. The 5' cap of an mRNA comprises an N7-methylated guanosine residue joined to the 5'-most residue of the mRNA via a 5'-5' triphosphate linkage. The 5' cap region of an mRNA is considered to include the 5' cap structure itself as well as the first 50 nucleotides adjacent to the cap.

30 The 5' cap region may also be a preferred target region.

Although some eukaryotic mRNA transcripts are directly translated, many contain one or more regions, known as "introns," which are excised from a transcript before it is translated. The remaining (and therefore translated) regions are known as "exons" and are spliced together to

-7-

form a continuous mRNA sequence. mRNA splice sites, i.e., intron-exon junctions, may also be preferred target regions, and are particularly useful in situations where aberrant splicing is implicated in disease, or where an overproduction of a particular mRNA splice product is implicated in disease. Aberrant fusion junctions due to rearrangements or deletions are also preferred targets. It has also been found that introns can also be effective, and therefore preferred, target regions for antisense compounds targeted, for example, to DNA or pre-mRNA.

Once one or more target sites have been identified, oligonucleotides are chosen which are sufficiently complementary to the target, i.e., hybridize sufficiently well and with sufficient specificity, to give the desired effect.

In the context of this invention, "hybridization" means hydrogen bonding, which may be Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen bonding, between complementary nucleoside or nucleotide bases. For example, adenine and thymine are complementary nucleobases which pair through the formation of hydrogen bonds.

"Complementary," as used herein, refers to the capacity for precise pairing between two nucleotides. For example, if a nucleotide at a certain position of an oligonucleotide is capable of hydrogen bonding with a nucleotide at the same position of a DNA or RNA molecule, then the oligonucleotide and the DNA or RNA are considered to be complementary to each other at that position. The oligonucleotide and the DNA or RNA are complementary to each other when a sufficient number of corresponding positions in each molecule are occupied by nucleotides which can hydrogen bond with each other. Thus, "specifically hybridizable" and "complementary" are terms which are used to indicate a sufficient degree of complementarity or precise pairing such that stable and specific binding occurs between the

-8-

oligonucleotide and the DNA or RNA target. It is understood in the art that the sequence of an antisense compound need not be 100% complementary to that of its target nucleic acid to be specifically hybridizable. An
5 antisense compound is specifically hybridizable when binding of the compound to the target DNA or RNA molecule interferes with the normal function of the target DNA or RNA to cause a loss of utility, and there is a sufficient degree of complementarity to avoid non-specific binding of
10 the antisense compound to non-target sequences under conditions in which specific binding is desired, i.e., under physiological conditions in the case of in vivo assays or therapeutic treatment, and in the case of in vitro assays, under conditions in which the assays are
15 performed.

Antisense compounds are commonly used as research reagents and diagnostics. For example, antisense oligonucleotides, which are able to inhibit gene expression with exquisite specificity, are often used by those of
20 ordinary skill to elucidate the function of particular genes. Antisense compounds are also used, for example, to distinguish between functions of various members of a biological pathway. Antisense modulation has, therefore, been harnessed for research use.

25 The specificity and sensitivity of antisense is also harnessed by those of skill in the art for therapeutic uses. Antisense oligonucleotides have been employed as therapeutic moieties in the treatment of disease states in animals and man. Antisense oligonucleotides have been
30 safely and effectively administered to humans and numerous clinical trials are presently underway. It is thus established that oligonucleotides can be useful therapeutic modalities that can be configured to be useful in treatment regimes for treatment of cells, tissues and animals,
35 especially humans.

-9-

In the context of this invention, the term "oligonucleotide" refers to an oligomer or polymer of ribonucleic acid (RNA) or deoxyribonucleic acid (DNA) or mimetics thereof. This term includes oligonucleotides

5 composed of naturally-occurring nucleobases, sugars and covalent internucleoside (backbone) linkages as well as oligonucleotides having non-naturally-occurring portions which function similarly. Such modified or substituted oligonucleotides are often preferred over native forms
10 because of desirable properties such as, for example, enhanced cellular uptake, enhanced affinity for nucleic acid target and increased stability in the presence of nucleases.

While antisense oligonucleotides are a preferred form
15 of antisense compound, the present invention comprehends other oligomeric antisense compounds, including but not limited to oligonucleotide mimetics such as are described below. The antisense compounds in accordance with this invention preferably comprise from about 8 to about 30
20 nucleobases. Particularly preferred are antisense oligonucleotides comprising from about 8 to about 30 nucleobases (i.e. from about 8 to about 30 linked nucleosides). As is known in the art, a nucleoside is a base-sugar combination. The base portion of the nucleoside
25 is normally a heterocyclic base. The two most common classes of such heterocyclic bases are the purines and the pyrimidines. Nucleotides are nucleosides that further include a phosphate group covalently linked to the sugar portion of the nucleoside. For those nucleosides that
30 include a pentofuranosyl sugar, the phosphate group can be linked to either the 2', 3' or 5' hydroxyl moiety of the sugar. In forming oligonucleotides, the phosphate groups covalently link adjacent nucleosides to one another to form a linear polymeric compound. In turn the respective ends
35 of this linear polymeric structure can be further joined to

-10-

form a circular structure, however, open linear structures are generally preferred. Within the oligonucleotide structure, the phosphate groups are commonly referred to as forming the internucleoside backbone of the
5 oligonucleotide. The normal linkage or backbone of RNA and DNA is a 3' to 5' phosphodiester linkage.

Specific examples of preferred antisense compounds useful in this invention include oligonucleotides containing modified backbones or non-natural
10 internucleoside linkages. As defined in this specification, oligonucleotides having modified backbones include those that retain a phosphorus atom in the backbone and those that do not have a phosphorus atom in the backbone. For the purposes of this specification, and as
15 sometimes referenced in the art, modified oligonucleotides that do not have a phosphorus atom in their internucleoside backbone can also be considered to be oligonucleosides.

Preferred modified oligonucleotide backbones include, for example, phosphorothioates, chiral phosphorothioates,
20 phosphorodithioates, phosphotriesters, aminoalkyl-phosphotriesters, methyl and other alkyl phosphonates including 3'-alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates including 3'-amino phosphoramidate and aminoalkylphosphoramidates,
25 thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, and boranophosphates having normal 3'-5' linkages, 2'-5' linked analogs of these, and those having inverted polarity wherein the adjacent pairs of nucleoside units are linked 3'-5' to 5'-3' or 2'-5' to
30 5'-2'. Various salts, mixed salts and free acid forms are also included.

Representative United States patents that teach the preparation of the above phosphorus-containing linkages include, but are not limited to, U.S.: 3,687,808;
35 4,469,863; 4,476,301; 5,023,243; 5,177,196; 5,188,897;

-11-

5,264,423; 5,276,019; 5,278,302; 5,286,717; 5,321,131;
5,399,676; 5,405,939; 5,453,496; 5,455,233; 5,466,677;
5,476,925; 5,519,126; 5,536,821; 5,541,306; 5,550,111;
5,563,253; 5,571,799; 5,587,361; and 5,625,050, certain of
5 which are commonly owned with this application, and each of
which is herein incorporated by reference.

Preferred modified oligonucleotide backbones that do
not include a phosphorus atom therein have backbones that
are formed by short chain alkyl or cycloalkyl
10 internucleoside linkages, mixed heteroatom and alkyl or
cycloalkyl internucleoside linkages, or one or more short
chain heteroatomic or heterocyclic internucleoside
linkages. These include those having morpholino linkages
(formed in part from the sugar portion of a nucleoside);
15 siloxane backbones; sulfide, sulfoxide and sulfone
backbones; formacetyl and thioformacetyl backbones;
methylene formacetyl and thioformacetyl backbones; alkene
containing backbones; sulfamate backbones; methyleneimino
and methylenehydrazino backbones; sulfonate and sulfonamide
20 backbones; amide backbones; and others having mixed N, O, S
and CH₂ component parts.

Representative United States patents that teach the
preparation of the above oligonucleosides include, but are
not limited to, U.S.: 5,034,506; 5,166,315; 5,185,444;
25 5,214,134; 5,216,141; 5,235,033; 5,264,562; 5,264,564;
5,405,938; 5,434,257; 5,466,677; 5,470,967; 5,489,677;
5,541,307; 5,561,225; 5,596,086; 5,602,240; 5,610,289;
5,602,240; 5,608,046; 5,610,289; 5,618,704; 5,623,070;
5,663,312; 5,633,360; 5,677,437; and 5,677,439, certain of
30 which are commonly owned with this application, and each of
which is herein incorporated by reference.

In other preferred oligonucleotide mimetics, both the
sugar and the internucleoside linkage, i.e., the backbone,
of the nucleotide units are replaced with novel groups.
35 The base units are maintained for hybridization with an

-12-

appropriate nucleic acid target compound. One such oligomeric compound, an oligonucleotide mimetic that has been shown to have excellent hybridization properties, is referred to as a peptide nucleic acid (PNA). In PNA

- 5 compounds, the sugar-backbone of an oligonucleotide is replaced with an amide containing backbone, in particular an aminoethylglycine backbone. The nucleobases are retained and are bound directly or indirectly to aza nitrogen atoms of the amide portion of the backbone.
- 10 Representative United States patents that teach the preparation of PNA compounds include, but are not limited to, U.S.: 5,539,082; 5,714,331; and 5,719,262, each of which is herein incorporated by reference. Further teaching of PNA compounds can be found in Nielsen et al., *Science*,
- 15 1991, 254, 1497-1500.

Most preferred embodiments of the invention are oligonucleotides with phosphorothioate backbones and oligonucleosides with heteroatom backbones, and in particular $-\text{CH}_2-\text{NH}-\text{O}-\text{CH}_2-$, $-\text{CH}_2-\text{N}(\text{CH}_3)-\text{O}-\text{CH}_2-$ [known as a

20 methylene (methylimino) or MMI backbone], $-\text{CH}_2-\text{O}-\text{N}(\text{CH}_3)-\text{CH}_2-$, $-\text{CH}_2-\text{N}(\text{CH}_3)-\text{N}(\text{CH}_3)-\text{CH}_2-$ and $-\text{O}-\text{N}(\text{CH}_3)-\text{CH}_2-\text{CH}_2-$ [wherein the native phosphodiester backbone is represented as $-\text{O}-\text{P}-\text{O}-\text{CH}_2-$] of the above referenced U.S. patent 5,489,677, and the amide backbones of the above referenced U.S. patent

25 5,602,240. Also preferred are oligonucleotides having morpholino backbone structures of the above-referenced U.S. patent 5,034,506.

Modified oligonucleotides may also contain one or more substituted sugar moieties. Preferred oligonucleotides

30 comprise one of the following at the 2' position: OH; F; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; O-, S- or N-alkynyl; or O-alkyl-O-alkyl, wherein the alkyl, alkenyl and alkynyl may be substituted or unsubstituted C_1 to C_{10} alkyl or C_2 to C_{10} alkenyl and alkynyl. Particularly preferred are

35 $\text{O}[(\text{CH}_2)_n\text{O}]_m\text{CH}_3$, $\text{O}(\text{CH}_2)_n\text{OCH}_3$, $\text{O}(\text{CH}_2)_n\text{NH}_2$, $\text{O}(\text{CH}_2)_n\text{CH}_3$, $\text{O}(\text{CH}_2)_n\text{ONH}_2$,

-13-

and $O(CH_2)_nON[(CH_2)_mCH_3]_2$, where n and m are from 1 to about 10. Other preferred oligonucleotides comprise one of the following at the 2' position: C_1 to C_{10} lower alkyl, substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH_3 , OCN, Cl, Br, CN, CF_3 , OCF_3 , $SOCH_3$, SO_2CH_3 , ONO_2 , NO_2 , N_3 , NH_2 , heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving the pharmacokinetic properties of an oligonucleotide, or a group for improving the pharmacodynamic properties of an oligonucleotide, and other substituents having similar properties. A preferred modification includes 2'-methoxyethoxy (2'-O- $CH_2CH_2OCH_3$, also known as 2'-O-(2-methoxyethyl) or 2'-MOE) (Martin et al., *Helv. Chim. Acta*, 1995, 78, 486-504) i.e., an alkoxyalkoxy group. A further preferred modification includes 2'-dimethylaminooxyethoxy, i.e., a $O(CH_2)_2ON(CH_3)_2$ group, also known as 2'-DMAOE, as described in examples hereinbelow.

Other preferred modifications include 2'-methoxy (2'-O- CH_3), 2'-aminopropoxy (2'-O $CH_2CH_2CH_2NH_2$) and 2'-fluoro (2'-F). Similar modifications may also be made at other positions on the oligonucleotide, particularly the 3' position of the sugar on the 3' terminal nucleotide or in 2'-5' linked oligonucleotides and the 5' position of 5' terminal nucleotide. Oligonucleotides may also have sugar mimetics such as cyclobutyl moieties in place of the pentofuranosyl sugar. Representative United States patents that teach the preparation of such modified sugar structures include, but are not limited to, U.S.:

4,981,957; 5,118,800; 5,319,080; 5,359,044; 5,393,878; 5,446,137; 5,466,786; 5,514,785; 5,519,134; 5,567,811; 5,576,427; 5,591,722; 5,597,909; 5,610,300; 5,627,053; 5,639,873; 5,646,265; 5,658,873; 5,670,633; and 5,700,920, certain of which are commonly owned with the instant

-14-

application, and each of which is herein incorporated by reference in its entirety.

Oligonucleotides may also include nucleobase (often referred to in the art simply as "base") modifications or substitutions. As used herein, "unmodified" or "natural" nucleobases include the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C) and uracil (U). Modified nucleobases include other synthetic and natural nucleobases such as 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl uracil and cytosine, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines and guanines, 5-halo particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-deazaadenine and 3-deazaguanine and 3-deazaadenine. Further nucleobases include those disclosed in United States Patent No. 3,687,808, those disclosed in *The Concise Encyclopedia Of Polymer Science And Engineering*, pages 858-859, Kroschwitz, J.I., ed. John Wiley & Sons, 1990, those disclosed by Englisch et al., *Angewandte Chemie*, International Edition, 1991, 30, 613, and those disclosed by Sanghvi, Y.S., Chapter 15, *Antisense Research and Applications*, pages 289-302, Crooke, S.T. and Lebleu, B. , ed., CRC Press, 1993. Certain of these nucleobases are particularly useful for increasing the binding affinity of the oligomeric compounds of the invention. These include 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and O-6 substituted purines,

-15-

including 2-aminopropyladenine, 5-propynyluracil and 5-propynylcytosine. 5-methylcytosine substitutions have been shown to increase nucleic acid duplex stability by 0.6-1.2°C (Sanghvi, Y.S., Crooke, S.T. and Lebleu, B., eds.,

5 *Antisense Research and Applications*, CRC Press, Boca Raton, 1993, pp. 276-278) and are presently preferred base substitutions, even more particularly when combined with 2'-O-methoxyethyl sugar modifications.

Representative United States patents that teach the
10 preparation of certain of the above noted modified nucleobases as well as other modified nucleobases include, but are not limited to, the above noted U.S. 3,687,808, as well as U.S.: 4,845,205; 5,130,302; 5,134,066; 5,175,273; 5,367,066; 5,432,272; 5,457,187; 5,459,255; 5,484,908;
15 5,502,177; 5,525,711; 5,552,540; 5,587,469; 5,594,121, 5,596,091; 5,614,617; and 5,681,941, certain of which are commonly owned with the instant application, and each of which is herein incorporated by reference, and United States patent 5,750,692, which is commonly owned with the
20 instant application and also herein incorporated by reference.

Another modification of the oligonucleotides of the invention involves chemically linking to the oligonucleotide one or more moieties or conjugates which
25 enhance the activity, cellular distribution or cellular uptake of the oligonucleotide. Such moieties include but are not limited to lipid moieties such as a cholesterol moiety (Letsinger et al., *Proc. Natl. Acad. Sci. USA*, 1989, 86, 6553-6556), cholic acid (Manoharan et al., *Bioorg. Med. Chem. Let.*, 1994, 4, 1053-1060), a thioether, e.g., hexyl-S-tritylthiol (Manoharan et al., *Ann. N.Y. Acad. Sci.*,
30 1992, 660, 306-309; Manoharan et al., *Bioorg. Med. Chem. Let.*, 1993, 3, 2765-2770), a thiocholesterol (Oberhauser et al., *Nucl. Acids Res.*, 1992, 20, 533-538), an aliphatic

20492650

-16-

chain, e.g., dodecandiol or undecyl residues (Saison-Behmoaras et al., *EMBO J.*, 1991, 10, 1111-1118; Kabanov et al., *FEBS Lett.*, 1990, 259, 327-330; Svinarchuk et al., *Biochimie*, 1993, 75, 49-54), a phospholipid, e.g., di-
5 hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate (Manoharan et al., *Tetrahedron Lett.*, 1995, 36, 3651-3654; Shea et al., *Nucl. Acids Res.*, 1990, 18, 3777-3783), a polyamine or a polyethylene glycol chain (Manoharan et al., *Nucleosides &*
10 *Nucleotides*, 1995, 14, 969-973), or adamantane acetic acid (Manoharan et al., *Tetrahedron Lett.*, 1995, 36, 3651-3654), a palmityl moiety (Mishra et al., *Biochim. Biophys. Acta*, 1995, 1264, 229-237), or an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety (Crooke et al., *J.*
15 *Pharmacol. Exp. Ther.*, 1996, 277, 923-937.

Representative United States patents that teach the preparation of such oligonucleotide conjugates include, but are not limited to, U.S.: 4,828,979; 4,948,882; 5,218,105; 5,525,465; 5,541,313; 5,545,730; 5,552,538; 5,578,717;
20 5,580,731; 5,580,731; 5,591,584; 5,109,124; 5,118,802; 5,138,045; 5,414,077; 5,486,603; 5,512,439; 5,578,718; 5,608,046; 4,587,044; 4,605,735; 4,667,025; 4,762,779; 4,789,737; 4,824,941; 4,835,263; 4,876,335; 4,904,582; 4,958,013; 5,082,830; 5,112,963; 5,214,136; 5,082,830;
25 5,112,963; 5,214,136; 5,245,022; 5,254,469; 5,258,506; 5,262,536; 5,272,250; 5,292,873; 5,317,098; 5,371,241; 5,391,723; 5,416,203; 5,451,463; 5,510,475; 5,512,667; 5,514,785; 5,565,552; 5,567,810; 5,574,142; 5,585,481; 5,587,371; 5,595,726; 5,597,696; 5,599,923; 5,599,928 and
30 5,688,941, certain of which are commonly owned with the instant application, and each of which is herein incorporated by reference.

It is not necessary for all positions in a given compound to be uniformly modified, and in fact more than

-17-

one of the aforementioned modifications may be incorporated in a single compound or even at a single nucleoside within an oligonucleotide. The present invention also includes antisense compounds which are chimeric compounds.

- 5 "Chimeric" antisense compounds or "chimeras," in the context of this invention, are antisense compounds, particularly oligonucleotides, which contain two or more chemically distinct regions, each made up of at least one monomer unit, i.e., a nucleotide in the case of an
- 10 oligonucleotide compound. These oligonucleotides typically contain at least one region wherein the oligonucleotide is modified so as to confer upon the oligonucleotide increased resistance to nuclease degradation, increased cellular uptake, and/or increased binding affinity for the target
- 15 nucleic acid. An additional region of the oligonucleotide may serve as a substrate for enzymes capable of cleaving RNA:DNA or RNA:RNA hybrids. By way of example, RNase H is a cellular endonuclease which cleaves the RNA strand of an RNA:DNA duplex. Activation of RNase H, therefore, results
- 20 in cleavage of the RNA target, thereby greatly enhancing the efficiency of oligonucleotide inhibition of gene expression. Consequently, comparable results can often be obtained with shorter oligonucleotides when chimeric oligonucleotides are used, compared to phosphorothioate
- 25 deoxyoligonucleotides hybridizing to the same target region. Cleavage of the RNA target can be routinely detected by gel electrophoresis and, if necessary, associated nucleic acid hybridization techniques known in the art.
- 30 Chimeric antisense compounds of the invention may be formed as composite structures of two or more oligonucleotides, modified oligonucleotides, oligonucleosides and/or oligonucleotide mimetics as described above. Such compounds have also been referred to
- 35 in the art as hybrids or gapmers. Representative United

-18-

States patents that teach the preparation of such hybrid structures include, but are not limited to, U.S.:

5,013,830; 5,149,797; 5,220,007; 5,256,775; 5,366,878;
5,403,711; 5,491,133; 5,565,350; 5,623,065; 5,652,355;

5 5,652,356; and 5,700,922, certain of which are commonly owned with the instant application, and each of which is herein incorporated by reference in its entirety.

The antisense compounds used in accordance with this invention may be conveniently and routinely made through
10 the well-known technique of solid phase synthesis. Equipment for such synthesis is sold by several vendors including, for example, Applied Biosystems (Foster City, CA). Any other means for such synthesis known in the art may additionally or alternatively be employed. It is well
15 known to use similar techniques to prepare oligonucleotides such as the phosphorothioates and alkylated derivatives.

The antisense compounds of the invention are synthesized in vitro and do not include antisense compositions of biological origin, or genetic vector
20 constructs designed to direct the in vivo synthesis of antisense molecules.

The compounds of the invention may also be admixed, encapsulated, conjugated or otherwise associated with other molecules, molecule structures or mixtures of compounds, as
25 for example, liposomes, receptor targeted molecules, oral, rectal, topical or other formulations, for assisting in uptake, distribution and/or absorption. Representative United States patents that teach the preparation of such uptake, distribution and/or absorption assisting

30 formulations include, but are not limited to, U.S.:

5,108,921; 5,354,844; 5,416,016; 5,459,127; 5,521,291;
5,543,158; 5,547,932; 5,583,020; 5,591,721; 4,426,330;
4,534,899; 5,013,556; 5,108,921; 5,213,804; 5,227,170;
5,264,221; 5,356,633; 5,395,619; 5,416,016; 5,417,978;

35 5,462,854; 5,469,854; 5,512,295; 5,527,528; 5,534,259;

-19-

5,543,152; 5,556,948; 5,580,575; and 5,595,756, each of which is herein incorporated by reference.

The antisense compounds of the invention encompass any pharmaceutically acceptable salts, esters, or salts of such
5 esters, or any other compound which, upon administration to an animal including a human, is capable of providing (directly or indirectly) the biologically active metabolite or residue thereof. Accordingly, for example, the disclosure is also drawn to prodrugs and pharmaceutically
10 acceptable salts of the compounds of the invention, pharmaceutically acceptable salts of such prodrugs, and other bioequivalents.

The term "prodrug" indicates a therapeutic agent that is prepared in an inactive form that is converted to an
15 active form (i.e., drug) within the body or cells thereof by the action of endogenous enzymes or other chemicals and/or conditions. In particular, prodrug versions of the oligonucleotides of the invention are prepared as SATE [(S-acetyl-2-thioethyl) phosphate] derivatives according to
20 the methods disclosed in WO 93/24510 to Gosselin et al., published December 9, 1993 or in WO 94/26764 to Imbach et al.

The term "pharmaceutically acceptable salts" refers to physiologically and pharmaceutically acceptable salts of
25 the compounds of the invention: i.e., salts that retain the desired biological activity of the parent compound and do not impart undesired toxicological effects thereto.

Pharmaceutically acceptable base addition salts are formed with metals or amines, such as alkali and alkaline
30 earth metals or organic amines. Examples of metals used as cations are sodium, potassium, magnesium, calcium, and the like. Examples of suitable amines are N,N'-dibenzylethylenediamine, chloroprocaine, choline, diethanolamine, dicyclohexylamine, ethylenediamine,
35 N-methylglucamine, and procaine (see, for example, Berge et

-20-

al., "Pharmaceutical Salts," *J. of Pharma Sci.*, 1977, 66, 1-19). The base addition salts of said acidic compounds are prepared by contacting the free acid form with a sufficient amount of the desired base to produce the salt in the conventional manner. The free acid form may be regenerated by contacting the salt form with an acid and isolating the free acid in the conventional manner. The free acid forms differ from their respective salt forms somewhat in certain physical properties such as solubility in polar solvents, but otherwise the salts are equivalent to their respective free acid for purposes of the present invention. As used herein, a "pharmaceutical addition salt" includes a pharmaceutically acceptable salt of an acid form of one of the components of the compositions of the invention. These include organic or inorganic acid salts of the amines. Preferred acid salts are the hydrochlorides, acetates, salicylates, nitrates and phosphates. Other suitable pharmaceutically acceptable salts are well known to those skilled in the art and include basic salts of a variety of inorganic and organic acids, such as, for example, with inorganic acids, such as for example hydrochloric acid, hydrobromic acid, sulfuric acid or phosphoric acid; with organic carboxylic, sulfonic, sulfo or phospho acids or N-substituted sulfamic acids, for example acetic acid, propionic acid, glycolic acid, succinic acid, maleic acid, hydroxymaleic acid, methylemaleic acid, fumaric acid, malic acid, tartaric acid, lactic acid, oxalic acid, gluconic acid, glucaric acid, glucuronic acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, salicylic acid, 4-aminosalicylic acid, 2-phenoxybenzoic acid, 2-acetoxybenzoic acid, embonic acid, nicotinic acid or isonicotinic acid; and with amino acids, such as the 20 alpha-amino acids involved in the synthesis of proteins in nature, for example glutamic acid or aspartic acid, and also with phenylacetic acid,

-21-

methanesulfonic acid, ethanesulfonic acid,
2-hydroxyethanesulfonic acid, ethane-1,2-disulfonic acid,
benzenesulfonic acid, 4-methylbenzenesulfoic acid,
naphthalene-2-sulfonic acid, naphthalene-1,5-disulfonic
5 acid, 2- or 3-phosphoglycerate, glucose-6-phosphate,
N-cyclohexylsulfamic acid (with the formation of
cyclamates), or with other acid organic compounds, such as
ascorbic acid. Pharmaceutically acceptable salts of
compounds may also be prepared with a pharmaceutically
10 acceptable cation. Suitable pharmaceutically acceptable
cations are well known to those skilled in the art and
include alkaline, alkaline earth, ammonium and quaternary
ammonium cations. Carbonates or hydrogen carbonates are
also possible.

15 For oligonucleotides, preferred examples of
pharmaceutically acceptable salts include but are not
limited to (a) salts formed with cations such as sodium,
potassium, ammonium, magnesium, calcium, polyamines such as
spermine and spermidine, etc.; (b) acid addition salts
20 formed with inorganic acids, for example hydrochloric acid,
hydrobromic acid, sulfuric acid, phosphoric acid, nitric
acid and the like; (c) salts formed with organic acids
such as, for example, acetic acid, oxalic acid, tartaric
acid, succinic acid, maleic acid, fumaric acid, gluconic
25 acid, citric acid, malic acid, ascorbic acid, benzoic acid,
tannic acid, palmitic acid, alginic acid, polyglutamic
acid, naphthalenesulfonic acid, methanesulfonic acid,
p-toluenesulfonic acid, naphthalenedisulfonic acid,
polygalacturonic acid, and the like; and (d) salts formed
30 from elemental anions such as chlorine, bromine, and
iodine.

The antisense compounds of the present invention can
be utilized for diagnostics, therapeutics, prophylaxis and
as research reagents and kits. For therapeutics, an
35 animal, preferably a human, suspected of having a disease

-22-

or disorder which can be treated by modulating the expression of SRA is treated by administering antisense compounds in accordance with this invention. The compounds of the invention can be utilized in pharmaceutical

5 compositions by adding an effective amount of an antisense compound to a suitable pharmaceutically acceptable diluent or carrier. Use of the antisense compounds and methods of the invention may also be useful prophylactically, e.g., to prevent or delay infection, inflammation or tumor
10 formation, for example.

The antisense compounds of the invention are useful for research and diagnostics, because these compounds hybridize to SRA or nucleic acids encoding SRA, enabling sandwich and other assays to easily be constructed to
15 exploit this fact. Hybridization of the antisense oligonucleotides of the invention with SRA or a nucleic acid encoding SRA can be detected by means known in the art. Such means may include conjugation of an enzyme to the oligonucleotide, radiolabelling of the oligonucleotide
20 or any other suitable detection means. Kits using such detection means for detecting the level of SRA in a sample may also be prepared.

The present invention also includes pharmaceutical compositions and formulations which include the antisense
25 compounds of the invention. The pharmaceutical compositions of the present invention may be administered in a number of ways depending upon whether local or systemic treatment is desired and upon the area to be treated. Administration may be topical (including
30 ophthalmic and to mucous membranes including vaginal and rectal delivery), pulmonary, e.g., by inhalation or insufflation of powders or aerosols, including by nebulizer; intratracheal, intranasal, epidermal and transdermal), oral or parenteral. Parenteral
35 administration includes intravenous, intraarterial,

-23-

subcutaneous, intraperitoneal or intramuscular injection or infusion; or intracranial, e.g., intrathecal or intraventricular, administration. Oligonucleotides with at least one 2'-O-methoxyethyl modification are believed to be particularly useful for oral administration.

Pharmaceutical compositions and formulations for topical administration may include transdermal patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable. Coated condoms, gloves and the like may also be useful.

Compositions and formulations for oral administration include powders or granules, suspensions or solutions in water or non-aqueous media, capsules, sachets or tablets. Thickeners, flavoring agents, diluents, emulsifiers, dispersing aids or binders may be desirable.

Compositions and formulations for parenteral, intrathecal or intraventricular administration may include sterile aqueous solutions which may also contain buffers, diluents and other suitable additives such as, but not limited to, penetration enhancers, carrier compounds and other pharmaceutically acceptable carriers or excipients.

Pharmaceutical compositions of the present invention include, but are not limited to, solutions, emulsions, and liposome-containing formulations. These compositions may be generated from a variety of components that include, but are not limited to, preformed liquids, self-emulsifying solids and self-emulsifying semisolids.

The pharmaceutical formulations of the present invention, which may conveniently be presented in unit dosage form, may be prepared according to conventional techniques well known in the pharmaceutical industry. Such techniques include the step of bringing into association the active ingredients with the pharmaceutical carrier(s)

-24-

or excipient(s). In general the formulations are prepared by uniformly and intimately bringing into association the active ingredients with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

The compositions of the present invention may be formulated into any of many possible dosage forms such as, but not limited to, tablets, capsules, liquid syrups, soft gels, suppositories, and enemas. The compositions of the present invention may also be formulated as suspensions in aqueous, non-aqueous or mixed media. Aqueous suspensions may further contain substances which increase the viscosity of the suspension including, for example, sodium carboxymethylcellulose, sorbitol and/or dextran. The suspension may also contain stabilizers.

In one embodiment of the present invention the pharmaceutical compositions may be formulated and used as foams. Pharmaceutical foams include formulations such as, but not limited to, emulsions, microemulsions, creams, jellies and liposomes. While basically similar in nature these formulations vary in the components and the consistency of the final product. The preparation of such compositions and formulations is generally known to those skilled in the pharmaceutical and formulation arts and may be applied to the formulation of the compositions of the present invention.

Emulsions

The compositions of the present invention may be prepared and formulated as emulsions. Emulsions are typically heterogenous systems of one liquid dispersed in another in the form of droplets usually exceeding 0.1 μm in diameter. (Idson, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199; Rosoff, in

-25-

Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., Volume 1, p. 245; Block in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 2, p. 335; Higuchi et al., in *Remington's Pharmaceutical Sciences*, Mack Publishing Co., Easton, PA, 1985, p. 301). Emulsions are often biphasic systems comprising of two immiscible liquid phases intimately mixed and dispersed with each other. In general, emulsions may be either water-in-oil (w/o) or of the oil-in-water (o/w) variety. When an aqueous phase is finely divided into and dispersed as minute droplets into a bulk oily phase the resulting composition is called a water-in-oil (w/o) emulsion. Alternatively, when an oily phase is finely divided into and dispersed as minute droplets into a bulk aqueous phase the resulting composition is called an oil-in-water (o/w) emulsion. Emulsions may contain additional components in addition to the dispersed phases and the active drug which may be present as a solution in either the aqueous phase, oily phase or itself as a separate phase. Pharmaceutical excipients such as emulsifiers, stabilizers, dyes, and anti-oxidants may also be present in emulsions as needed. Pharmaceutical emulsions may also be multiple emulsions that are comprised of more than two phases such as, for example, in the case of oil-in-water-in-oil (o/w/o) and water-in-oil-in-water (w/o/w) emulsions. Such complex formulations often provide certain advantages that simple binary emulsions do not. Multiple emulsions in which individual oil droplets of an o/w emulsion enclose small water droplets constitute a w/o/w emulsion. Likewise a system of oil droplets enclosed in globules of water stabilized in an oily continuous provides an o/w/o emulsion.

-26-

Emulsions are characterized by little or no thermodynamic stability. Often, the dispersed or discontinuous phase of the emulsion is well dispersed into the external or continuous phase and maintained in this form through the means of emulsifiers or the viscosity of the formulation. Either of the phases of the emulsion may be a semisolid or a solid, as is the case of emulsion-style ointment bases and creams. Other means of stabilizing emulsions entail the use of emulsifiers that may be incorporated into either phase of the emulsion. Emulsifiers may broadly be classified into four categories: synthetic surfactants, naturally occurring emulsifiers, absorption bases, and finely dispersed solids (Idson, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199).

Synthetic surfactants, also known as surface active agents, have found wide applicability in the formulation of emulsions and have been reviewed in the literature (Rieger, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 285; Idson, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), Marcel Dekker, Inc., New York, N.Y., 1988, volume 1, p. 199). Surfactants are typically amphiphilic and comprise a hydrophilic and a hydrophobic portion. The ratio of the hydrophilic to the hydrophobic nature of the surfactant has been termed the hydrophile/lipophile balance (HLB) and is a valuable tool in categorizing and selecting surfactants in the preparation of formulations. Surfactants may be classified into different classes based on the nature of the hydrophilic group: nonionic, anionic, cationic and amphoteric (Rieger, in *Pharmaceutical Dosage Forms*,

-27-

Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 285).

Naturally occurring emulsifiers used in emulsion formulations include lanolin, beeswax, phosphatides, lecithin and acacia. Absorption bases possess hydrophilic properties such that they can soak up water to form w/o emulsions yet retain their semisolid consistencies, such as anhydrous lanolin and hydrophilic petrolatum. Finely divided solids have also been used as good emulsifiers especially in combination with surfactants and in viscous preparations. These include polar inorganic solids, such as heavy metal hydroxides, nonswelling clays such as bentonite, attapulgite, hectorite, kaolin, montmorillonite, colloidal aluminum silicate and colloidal magnesium aluminum silicate, pigments and nonpolar solids such as carbon or glyceryl tristearate.

A large variety of non-emulsifying materials are also included in emulsion formulations and contribute to the properties of emulsions. These include fats, oils, waxes, fatty acids, fatty alcohols, fatty esters, humectants, hydrophilic colloids, preservatives and antioxidants (Block, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 335; Idson, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199).

Hydrophilic colloids or hydrocolloids include naturally occurring gums and synthetic polymers such as polysaccharides (for example, acacia, agar, alginic acid, carrageenan, guar gum, karaya gum, and tragacanth), cellulose derivatives (for example, carboxymethylcellulose and carboxypropylcellulose), and synthetic polymers (for example, carbomers, cellulose ethers, and carboxyvinyl polymers). These disperse or swell in water to form colloidal solutions that stabilize emulsions by forming

-28-

strong interfacial films around the dispersed-phase droplets and by increasing the viscosity of the external phase.

Since emulsions often contain a number of ingredients such as carbohydrates, proteins, sterols and phosphatides that may readily support the growth of microbes, these formulations often incorporate preservatives. Commonly used preservatives included in emulsion formulations include methyl paraben, propyl paraben, quaternary ammonium salts, benzalkonium chloride, esters of p-hydroxybenzoic acid, and boric acid. Antioxidants are also commonly added to emulsion formulations to prevent deterioration of the formulation. Antioxidants used may be free radical scavengers such as tocopherols, alkyl gallates, butylated hydroxyanisole, butylated hydroxytoluene, or reducing agents such as ascorbic acid and sodium metabisulfite, and antioxidant synergists such as citric acid, tartaric acid, and lecithin.

The application of emulsion formulations via dermatological, oral and parenteral routes and methods for their manufacture have been reviewed in the literature (Idson, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199). Emulsion formulations for oral delivery have been very widely used because of reasons of ease of formulation, efficacy from an absorption and bioavailability standpoint. (Rosoff, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 245; Idson, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199). Mineral-oil base laxatives, oil-soluble vitamins and high fat nutritive preparations are among the materials that have commonly been administered orally as o/w emulsions.

-29-

In one embodiment of the present invention, the compositions of oligonucleotides and nucleic acids are formulated as microemulsions. A microemulsion may be defined as a system of water, oil and amphiphile which is a single optically isotropic and thermodynamically stable liquid solution (Rosoff, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 245). Typically microemulsions are systems that are prepared by first dispersing an oil in an aqueous surfactant solution and then adding a sufficient amount of a fourth component, generally an intermediate chain-length alcohol to form a transparent system. Therefore, microemulsions have also been described as thermodynamically stable, isotropically clear dispersions of two immiscible liquids that are stabilized by interfacial films of surface-active molecules (Leung and Shah, in: *Controlled Release of Drugs: Polymers and Aggregate Systems*, Rosoff, M., Ed., 1989, VCH Publishers, New York, pages 185-215). Microemulsions commonly are prepared via a combination of three to five components that include oil, water, surfactant, cosurfactant and electrolyte. Whether the microemulsion is of the water-in-oil (w/o) or an oil-in-water (o/w) type is dependent on the properties of the oil and surfactant used and on the structure and geometric packing of the polar heads and hydrocarbon tails of the surfactant molecules (Schott, in *Remington's Pharmaceutical Sciences*, Mack Publishing Co., Easton, PA, 1985, p. 271).

The phenomenological approach utilizing phase diagrams has been extensively studied and has yielded a comprehensive knowledge, to one skilled in the art, of how to formulate microemulsions (Rosoff, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 245;

-30-

Block, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 335). Compared to conventional emulsions, microemulsions offer the advantage of solubilizing water-insoluble drugs in a formulation of thermodynamically stable droplets that are formed spontaneously.

Surfactants used in the preparation of microemulsions include, but are not limited to, ionic surfactants, non-ionic surfactants, Brij 96, polyoxyethylene oleyl ethers, polyglycerol fatty acid esters, tetraglycerol monolaurate (ML310), tetraglycerol monooleate (MO310), hexaglycerol monooleate (PO310), hexaglycerol pentaoleate (PO500), decaglycerol monocaprates (MCA750), decaglycerol monooleate (MO750), decaglycerol sequioleate (SO750), decaglycerol decaoleate (DAO750), alone or in combination with cosurfactants. The cosurfactant, usually a short-chain alcohol such as ethanol, 1-propanol, and 1-butanol, serves to increase the interfacial fluidity by penetrating into the surfactant film and consequently creating a disordered film because of the void space generated among surfactant molecules. Microemulsions may, however, be prepared without the use of cosurfactants and alcohol-free self-emulsifying microemulsion systems are known in the art. The aqueous phase may typically be, but is not limited to, water, an aqueous solution of the drug, glycerol, PEG300, PEG400, polyglycerols, propylene glycols, and derivatives of ethylene glycol. The oil phase may include, but is not limited to, materials such as Captex 300, Captex 355, Capmul MCM, fatty acid esters, medium chain (C8-C12) mono, di, and tri-glycerides, polyoxyethylated glyceryl fatty acid esters, fatty alcohols, polyglycolized glycerides, saturated polyglycolized C8-C10 glycerides, vegetable oils and silicone oil.

-31-

Microemulsions are particularly of interest from the standpoint of drug solubilization and the enhanced absorption of drugs. Lipid based microemulsions (both o/w and w/o) have been proposed to enhance the oral bioavailability of drugs, including peptides (Constantinides et al., *Pharmaceutical Research*, 1994, 11, 1385-1390; Ritschel, *Meth. Find. Exp. Clin. Pharmacol.*, 1993, 13, 205). Microemulsions afford advantages of improved drug solubilization, protection of drug from enzymatic hydrolysis, possible enhancement of drug absorption due to surfactant-induced alterations in membrane fluidity and permeability, ease of preparation, ease of oral administration over solid dosage forms, improved clinical potency, and decreased toxicity (Constantinides et al., *Pharmaceutical Research*, 1994, 11, 1385; Ho et al., *J. Pharm. Sci.*, 1996, 85, 138-143). Often microemulsions may form spontaneously when their components are brought together at ambient temperature. This may be particularly advantageous when formulating thermolabile drugs, peptides or oligonucleotides. Microemulsions have also been effective in the transdermal delivery of active components in both cosmetic and pharmaceutical applications. It is expected that the microemulsion compositions and formulations of the present invention will facilitate the increased systemic absorption of oligonucleotides and nucleic acids from the gastrointestinal tract, as well as improve the local cellular uptake of oligonucleotides and nucleic acids within the gastrointestinal tract, vagina, buccal cavity and other areas of administration.

Microemulsions of the present invention may also contain additional components and additives such as sorbitan monostearate (Grill 3), Labrasol, and penetration enhancers to improve the properties of the formulation and

-32-

to enhance the absorption of the oligonucleotides and nucleic acids of the present invention. Penetration enhancers used in the microemulsions of the present invention may be classified as belonging to one of five broad categories - surfactants, fatty acids, bile salts, chelating agents, and non-chelating non-surfactants (Lee et al., *Critical Reviews in Therapeutic Drug Carrier Systems*, 1991, p. 92). Each of these classes has been discussed above.

10 Liposomes

There are many organized surfactant structures besides microemulsions that have been studied and used for the formulation of drugs. These include monolayers, micelles, bilayers and vesicles. Vesicles, such as liposomes, have attracted great interest because of their specificity and the duration of action they offer from the standpoint of drug delivery. As used in the present invention, the term "liposome" means a vesicle composed of amphiphilic lipids arranged in a spherical bilayer or bilayers.

20 Liposomes are unilamellar or multilamellar vesicles which have a membrane formed from a lipophilic material and an aqueous interior. The aqueous portion contains the composition to be delivered. Cationic liposomes possess the advantage of being able to fuse to the cell wall. Non-cationic liposomes, although not able to fuse as efficiently with the cell wall, are taken up by macrophages *in vivo*.

In order to cross intact mammalian skin, lipid vesicles must pass through a series of fine pores, each with a diameter less than 50 nm, under the influence of a suitable transdermal gradient. Therefore, it is desirable to use a liposome which is highly deformable and able to pass through such fine pores.

Further advantages of liposomes include; liposomes obtained from natural phospholipids are biocompatible and

-33-

biodegradable; liposomes can incorporate a wide range of water and lipid soluble drugs; liposomes can protect encapsulated drugs in their internal compartments from metabolism and degradation (Rosoff, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 245). Important considerations in the preparation of liposome formulations are the lipid surface charge, vesicle size and the aqueous volume of the liposomes.

10 Liposomes are useful for the transfer and delivery of active ingredients to the site of action. Because the liposomal membrane is structurally similar to biological membranes, when liposomes are applied to a tissue, the liposomes start to merge with the cellular membranes. As
15 the merging of the liposome and cell progresses, the liposomal contents are emptied into the cell where the active agent may act.

Liposomal formulations have been the focus of extensive investigation as the mode of delivery for many
20 drugs. There is growing evidence that for topical administration, liposomes present several advantages over other formulations. Such advantages include reduced side-effects related to high systemic absorption of the administered drug, increased accumulation of the
25 administered drug at the desired target, and the ability to administer a wide variety of drugs, both hydrophilic and hydrophobic, into the skin.

Several reports have detailed the ability of liposomes to deliver agents including high-molecular weight DNA into
30 the skin. Compounds including analgesics, antibodies, hormones and high-molecular weight DNAs have been administered to the skin. The majority of applications resulted in the targeting of the upper epidermis.

Liposomes fall into two broad classes. Cationic
35 liposomes are positively charged liposomes which interact

-34-

with the negatively charged DNA molecules to form a stable complex. The positively charged DNA/liposome complex binds to the negatively charged cell surface and is internalized in an endosome. Due to the acidic pH within the endosome, the liposomes are ruptured, releasing their contents into the cell cytoplasm (Wang et al., *Biochem. Biophys. Res. Commun.*, 1987, 147, 980-985).

Liposomes which are pH-sensitive or negatively-charged, entrap DNA rather than complex with it. Since both the DNA and the lipid are similarly charged, repulsion rather than complex formation occurs. Nevertheless, some DNA is entrapped within the aqueous interior of these liposomes. pH-sensitive liposomes have been used to deliver DNA encoding the thymidine kinase gene to cell monolayers in culture. Expression of the exogenous gene was detected in the target cells (Zhou et al., *Journal of Controlled Release*, 1992, 19, 269-274).

One major type of liposomal composition includes phospholipids other than naturally-derived phosphatidylcholine. Neutral liposome compositions, for example, can be formed from dimyristoyl phosphatidylcholine (DMPC) or dipalmitoyl phosphatidylcholine (DPPC). Anionic liposome compositions generally are formed from dimyristoyl phosphatidylglycerol, while anionic fusogenic liposomes are formed primarily from dioleoyl phosphatidylethanolamine (DOPE). Another type of liposomal composition is formed from phosphatidylcholine (PC) such as, for example, soybean PC, and egg PC. Another type is formed from mixtures of phospholipid and/or phosphatidylcholine and/or cholesterol.

Several studies have assessed the topical delivery of liposomal drug formulations to the skin. Application of liposomes containing interferon to guinea pig skin resulted in a reduction of skin herpes sores while delivery of interferon via other means (e.g. as a solution or as an emulsion) were ineffective (Weiner et al., *Journal of Drug*

-35-

Targeting, 1992, 2, 405-410). Further, an additional study tested the efficacy of interferon administered as part of a liposomal formulation to the administration of interferon using an aqueous system, and concluded that the liposomal
5 formulation was superior to aqueous administration (du Plessis et al., *Antiviral Research*, 1992, 18, 259-265).

Non-ionic liposomal systems have also been examined to determine their utility in the delivery of drugs to the skin, in particular systems comprising non-ionic surfactant
10 and cholesterol. Non-ionic liposomal formulations comprising Novasome™ I (glyceryl dilaurate/cholesterol/polyoxyethylene-10-stearyl ether) and Novasome™ II (glyceryl distearate/cholesterol/polyoxyethylene-10-stearyl ether) were used to
15 deliver cyclosporin-A into the dermis of mouse skin. Results indicated that such non-ionic liposomal systems were effective in facilitating the deposition of cyclosporin-A into different layers of the skin (Hu et al. *S.T.P. Pharma. Sci.*, 1994, 4, 6, 466).

20 Liposomes also include "sterically stabilized" liposomes, a term which, as used herein, refers to liposomes comprising one or more specialized lipids that, when incorporated into liposomes, result in enhanced circulation lifetimes relative to liposomes lacking such
25 specialized lipids. Examples of sterically stabilized liposomes are those in which part of the vesicle-forming lipid portion of the liposome (A) comprises one or more glycolipids, such as monosialoganglioside G_{M1}, or (B) is derivatized with one or more hydrophilic polymers, such as
30 a polyethylene glycol (PEG) moiety. While not wishing to be bound by any particular theory, it is thought in the art that, at least for sterically stabilized liposomes containing gangliosides, sphingomyelin, or PEG-derivatized lipids, the enhanced circulation half-life of these
35 sterically stabilized liposomes derives from a reduced

-36-

uptake into cells of the reticuloendothelial system (RES) (Allen et al., *FEBS Letters*, 1987, 223, 42; Wu et al., *Cancer Research*, 1993, 53, 3765). Various liposomes

comprising one or more glycolipids are known in the art.

- 5 Papahadjopoulos et al. (*Ann. N.Y. Acad. Sci.*, 1987, 507, 64) reported the ability of monosialoganglioside G_{M1} , galactocerebroside sulfate and phosphatidylinositol to improve blood half-lives of liposomes. These findings were expounded upon by Gabizon et al. (*Proc. Natl. Acad. Sci.*
- 10 *U.S.A.*, 1988, 85, 6949). U.S. Patent No. 4,837,028 and WO 88/04924, both to Allen et al., disclose liposomes comprising (1) sphingomyelin and (2) the ganglioside G_{M1} or a galactocerebroside sulfate ester. U.S. Patent No. 5,543,152 (Webb et al.) discloses liposomes comprising
- 15 sphingomyelin. Liposomes comprising 1,2-*sn*-dimyristoylphosphatidylcholine are disclosed in WO 97/13499 (Lim et al.).

- Many liposomes comprising lipids derivatized with one or more hydrophilic polymers, and methods of preparation
- 20 thereof, are known in the art. Sunamoto et al. (*Bull. Chem. Soc. Jpn.*, 1980, 53, 2778) described liposomes comprising a nonionic detergent, 2C₁₂15G, that contains a PEG moiety. Illum et al. (*FEBS Lett.*, 1984, 167, 79) noted that hydrophilic coating of polystyrene particles with
- 25 polymeric glycols results in significantly enhanced blood half-lives. Synthetic phospholipids modified by the attachment of carboxylic groups of polyalkylene glycols (e.g., PEG) are described by Sears (U.S. Patent Nos. 4,426,330 and 4,534,899). Klibanov et al. (*FEBS Lett.*,
- 30 1990, 268, 235) described experiments demonstrating that liposomes comprising phosphatidylethanolamine (PE) derivatized with PEG or PEG stearate have significant increases in blood circulation half-lives. Blume et al. (*Biochimica et Biophysica Acta*, 1990, 1029, 91) extended

-37-

such observations to other PEG-derivatized phospholipids, e.g., DSPE-PEG, formed from the combination of distearoylphosphatidylethanolamine (DSPE) and PEG. Liposomes having covalently bound PEG moieties on their external surface are described in European Patent No. EP 0 445 131 B1 and WO 90/04384 to Fisher. Liposome compositions containing 1-20 mole percent of PE derivatized with PEG, and methods of use thereof, are described by Woodle et al. (U.S. Patent Nos. 5,013,556 and 5,356,633) and Martin et al. (U.S. Patent No. 5,213,804 and European Patent No. EP 0 496 813 B1). Liposomes comprising a number of other lipid-polymer conjugates are disclosed in WO 91/05545 and U.S. Patent No. 5,225,212 (both to Martin et al.) and in WO 94/20073 (Zalipsky et al.) Liposomes comprising PEG-modified ceramide lipids are described in WO 96/10391 (Choi et al.). U.S. Patent Nos. 5,540,935 (Miyazaki et al.) and 5,556,948 (Tagawa et al.) describe PEG-containing liposomes that can be further derivatized with functional moieties on their surfaces.

A limited number of liposomes comprising nucleic acids are known in the art. WO 96/40062 to Thierry et al. discloses methods for encapsulating high molecular weight nucleic acids in liposomes. U.S. Patent No. 5,264,221 to Tagawa et al. discloses protein-bonded liposomes and asserts that the contents of such liposomes may include an antisense RNA. U.S. Patent No. 5,665,710 to Rahman et al. describes certain methods of encapsulating oligodeoxynucleotides in liposomes. WO 97/04787 to Love et al. discloses liposomes comprising antisense oligonucleotides targeted to the raf gene.

Transfersomes are yet another type of liposomes, and are highly deformable lipid aggregates which are attractive candidates for drug delivery vehicles. Transfersomes may be described as lipid droplets which are so highly

-38-

deformable that they are easily able to penetrate through pores which are smaller than the droplet. Transfersomes are adaptable to the environment in which they are used, e.g. they are self-optimizing (adaptive to the shape of pores in the skin), self-repairing, frequently reach their targets without fragmenting, and often self-loading. To make transfersomes it is possible to add surface edge-activators, usually surfactants, to a standard liposomal composition. Transfersomes have been used to deliver serum albumin to the skin. The transfersome-mediated delivery of serum albumin has been shown to be as effective as subcutaneous injection of a solution containing serum albumin.

Surfactants find wide application in formulations such as emulsions (including microemulsions) and liposomes. The most common way of classifying and ranking the properties of the many different types of surfactants, both natural and synthetic, is by the use of the hydrophile/lipophile balance (HLB). The nature of the hydrophilic group (also known as the "head") provides the most useful means for categorizing the different surfactants used in formulations (Rieger, in *Pharmaceutical Dosage Forms*, Marcel Dekker, Inc., New York, NY, 1988, p. 285).

If the surfactant molecule is not ionized, it is classified as a nonionic surfactant. Nonionic surfactants find wide application in pharmaceutical and cosmetic products and are usable over a wide range of pH values. In general their HLB values range from 2 to about 18 depending on their structure. Nonionic surfactants include nonionic esters such as ethylene glycol esters, propylene glycol esters, glyceryl esters, polyglyceryl esters, sorbitan esters, sucrose esters, and ethoxylated esters. Nonionic alkanolamides and ethers such as fatty alcohol ethoxylates, propoxylated alcohols, and ethoxylated/propoxylated block polymers are also included in this class. The

polyoxyethylene surfactants are the most popular members of the nonionic surfactant class.

If the surfactant molecule carries a negative charge when it is dissolved or dispersed in water, the surfactant is classified as anionic. Anionic surfactants include carboxylates such as soaps, acyl lactylates, acyl amides of amino acids, esters of sulfuric acid such as alkyl sulfates and ethoxylated alkyl sulfates, sulfonates such as alkyl benzene sulfonates, acyl isethionates, acyl taurates and sulfosuccinates, and phosphates. The most important members of the anionic surfactant class are the alkyl sulfates and the soaps.

If the surfactant molecule carries a positive charge when it is dissolved or dispersed in water, the surfactant is classified as cationic. Cationic surfactants include quaternary ammonium salts and ethoxylated amines. The quaternary ammonium salts are the most used members of this class.

If the surfactant molecule has the ability to carry either a positive or negative charge, the surfactant is classified as amphoteric. Amphoteric surfactants include acrylic acid derivatives, substituted alkylamides, N-alkylbetaines and phosphatides.

The use of surfactants in drug products, formulations and in emulsions has been reviewed (Rieger, in *Pharmaceutical Dosage Forms*, Marcel Dekker, Inc., New York, NY, 1988, p. 285).

Penetration Enhancers

In one embodiment, the present invention employs various penetration enhancers to effect the efficient delivery of nucleic acids, particularly oligonucleotides, to the skin of animals. Most drugs are present in solution in both ionized and nonionized forms. However, usually only lipid soluble or lipophilic drugs readily cross cell membranes. It has been discovered that even non-lipophilic

-40-

drugs may cross cell membranes if the membrane to be crossed is treated with a penetration enhancer. In addition to aiding the diffusion of non-lipophilic drugs across cell membranes, penetration enhancers also enhance the permeability of lipophilic drugs.

Penetration enhancers may be classified as belonging to one of five broad categories, i.e., surfactants, fatty acids, bile salts, chelating agents, and non-chelating non-surfactants (Lee et al., *Critical Reviews in Therapeutic Drug Carrier Systems*, 1991, p.92). Each of the above mentioned classes of penetration enhancers are described below in greater detail.

Surfactants: In connection with the present invention, surfactants (or "surface-active agents") are chemical entities which, when dissolved in an aqueous solution, reduce the surface tension of the solution or the interfacial tension between the aqueous solution and another liquid, with the result that absorption of oligonucleotides through the mucosa is enhanced. In addition to bile salts and fatty acids, these penetration enhancers include, for example, sodium lauryl sulfate, polyoxyethylene-9-lauryl ether and polyoxyethylene-20-cetyl ether) (Lee et al., *Critical Reviews in Therapeutic Drug Carrier Systems*, 1991, p.92); and perfluorochemical emulsions, such as FC-43. Takahashi et al., *J. Pharm. Pharmacol.*, 1988, 40, 252).

Fatty acids: Various fatty acids and their derivatives which act as penetration enhancers include, for example, oleic acid, lauric acid, capric acid (n-decanoic acid), myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprate, tricaprate, monoolein (1-monooleoyl-rac-glycerol), dilaurin, caprylic acid, arachidonic acid, glycerol 1-monocaprate, 1-dodecylazacycloheptan-2-one, acylcarnitines, acylcholines,

-41-

C₁₋₁₀ alkyl esters thereof (e.g., methyl, isopropyl and t-butyl), and mono- and di-glycerides thereof (i.e., oleate, laurate, caprate, myristate, palmitate, stearate, linoleate, etc.) (Lee et al., *Critical Reviews in*

- 5 *Therapeutic Drug Carrier Systems*, 1991, p.92; Muranishi, *Critical Reviews in Therapeutic Drug Carrier Systems*, 1990, 7, 1-33; El Hariri et al., *J. Pharm. Pharmacol.*, 1992, 44, 651-654).

Bile salts: The physiological role of bile includes
10 the facilitation of dispersion and absorption of lipids and fat-soluble vitamins (Brunton, Chapter 38 in: Goodman & Gilman's *The Pharmacological Basis of Therapeutics*, 9th Ed., Hardman et al. Eds., McGraw-Hill, New York, 1996, pp. 934-935). Various natural bile salts, and their synthetic
15 derivatives, act as penetration enhancers. Thus the term "bile salts" includes any of the naturally occurring components of bile as well as any of their synthetic derivatives. The bile salts of the invention include, for example, cholic acid (or its pharmaceutically acceptable
20 sodium salt, sodium cholate), dehydrocholic acid (sodium dehydrocholate), deoxycholic acid (sodium deoxycholate), glucolic acid (sodium glucolate), glycholic acid (sodium glycocholate), glycodeoxycholic acid (sodium glycodeoxycholate), taurocholic acid (sodium taurocholate),
25 taurodeoxycholic acid (sodium taurodeoxycholate), chenodeoxycholic acid (sodium chenodeoxycholate), ursodeoxycholic acid (UDCA), sodium tauro-24,25-dihydrofusidate (STDHF), sodium glycodihydrofusidate and polyoxyethylene-9-lauryl ether (POE) (Lee et al., *Critical*
30 *Reviews in Therapeutic Drug Carrier Systems*, 1991, page 92; Swinyard, Chapter 39 In: *Remington's Pharmaceutical Sciences*, 18th Ed., Gennaro, ed., Mack Publishing Co., Easton, PA, 1990, pages 782-783; Muranishi, *Critical Reviews in Therapeutic Drug Carrier Systems*, 1990, 7, 1-33;

-42-

Yamamoto et al., *J. Pharm. Exp. Ther.*, 1992, 263, 25;
Yamashita et al., *J. Pharm. Sci.*, 1990, 79, 579-583).

Chelating Agents: Chelating agents, as used in connection with the present invention, can be defined as compounds that remove metallic ions from solution by forming complexes therewith, with the result that absorption of oligonucleotides through the mucosa is enhanced. With regards to their use as penetration enhancers in the present invention, chelating agents have the added advantage of also serving as DNase inhibitors, as most characterized DNA nucleases require a divalent metal ion for catalysis and are thus inhibited by chelating agents (Jarrett, *J. Chromatogr.*, 1993, 618, 315-339). Chelating agents of the invention include but are not limited to disodium ethylenediaminetetraacetate (EDTA), citric acid, salicylates (e.g., sodium salicylate, 5-methoxysalicylate and homovanilate), N-acyl derivatives of collagen, laureth-9 and N-amino acyl derivatives of beta-diketones (enamines) (Lee et al., *Critical Reviews in Therapeutic Drug Carrier Systems*, 1991, page 92; Muranishi, *Critical Reviews in Therapeutic Drug Carrier Systems*, 1990, 7, 1-33; Buur et al., *J. Control Rel.*, 1990, 14, 43-51).

Non-chelating non-surfactants: As used herein, non-chelating non-surfactant penetration enhancing compounds can be defined as compounds that demonstrate insignificant activity as chelating agents or as surfactants but that nonetheless enhance absorption of oligonucleotides through the alimentary mucosa (Muranishi, *Critical Reviews in Therapeutic Drug Carrier Systems*, 1990, 7, 1-33). This class of penetration enhancers include, for example, unsaturated cyclic ureas, 1-alkyl- and 1-alkenylazacycloalkanone derivatives (Lee et al., *Critical Reviews in Therapeutic Drug Carrier Systems*, 1991, page 92); and non-steroidal anti-inflammatory agents such as diclofenac

-43-

sodium, indomethacin and phenylbutazone (Yamashita et al., *J. Pharm. Pharmacol.*, 1987, 39, 621-626).

Agents that enhance uptake of oligonucleotides at the cellular level may also be added to the pharmaceutical and other compositions of the present invention. For example, cationic lipids, such as lipofectin (Junichi et al, U.S. Patent No. 5,705,188), cationic glycerol derivatives, and polycationic molecules, such as polylysine (Lollo et al., PCT Application WO 97/30731), are also known to enhance the cellular uptake of oligonucleotides.

Other agents may be utilized to enhance the penetration of the administered nucleic acids, including glycols such as ethylene glycol and propylene glycol, pyrrols such as 2-pyrrol, azones, and terpenes such as limonene and menthone.

Carriers

Certain compositions of the present invention also incorporate carrier compounds in the formulation. As used herein, "carrier compound" or "carrier" can refer to a nucleic acid, or analog thereof, which is inert (i.e., does not possess biological activity *per se*) but is recognized as a nucleic acid by *in vivo* processes that reduce the bioavailability of a nucleic acid having biological activity by, for example, degrading the biologically active nucleic acid or promoting its removal from circulation. The coadministration of a nucleic acid and a carrier compound, typically with an excess of the latter substance, can result in a substantial reduction of the amount of nucleic acid recovered in the liver, kidney or other extracirculatory reservoirs, presumably due to competition between the carrier compound and the nucleic acid for a common receptor. For example, the recovery of a partially phosphorothioate oligonucleotide in hepatic tissue can be reduced when it is coadministered with polyinosinic acid, dextran sulfate, polycytidic acid or 4-acetamido-

-44-

4'isothiocyano-stilbene-2,2'-disulfonic acid (Miyao et al., *Antisense Res. Dev.*, 1995, 5, 115-121; Takakura et al., *Antisense & Nucl. Acid Drug Dev.*, 1996, 6, 177-183).

Excipients

5 In contrast to a carrier compound, a "pharmaceutical carrier" or "excipient" is a pharmaceutically acceptable solvent, suspending agent or any other pharmacologically inert vehicle for delivering one or more nucleic acids to an animal. The excipient may be liquid or solid and is
10 selected, with the planned manner of administration in mind, so as to provide for the desired bulk, consistency, etc., when combined with a nucleic acid and the other components of a given pharmaceutical composition. Typical pharmaceutical carriers include, but are not limited to,
15 binding agents (e.g., pregelatinized maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose, etc.); fillers (e.g., lactose and other sugars, microcrystalline cellulose, pectin, gelatin, calcium sulfate, ethyl cellulose, polyacrylates or calcium hydrogen
20 phosphate, etc.); lubricants (e.g., magnesium stearate, talc, silica, colloidal silicon dioxide, stearic acid, metallic stearates, hydrogenated vegetable oils, corn starch, polyethylene glycols, sodium benzoate, sodium acetate, etc.); disintegrants (e.g., starch, sodium starch
25 glycolate, etc.); and wetting agents (e.g., sodium lauryl sulphate, etc.).

Pharmaceutically acceptable organic or inorganic excipient suitable for non-parenteral administration which do not deleteriously react with nucleic acids can also be
30 used to formulate the compositions of the present invention. Suitable pharmaceutically acceptable carriers include, but are not limited to, water, salt solutions, alcohols, polyethylene glycols, gelatin, lactose, amylose, magnesium stearate, talc, silicic acid, viscous paraffin,

-45-

hydroxymethylcellulose, polyvinylpyrrolidone and the like.

Formulations for topical administration of nucleic acids may include sterile and non-sterile aqueous solutions, non-aqueous solutions in common solvents such as
5 alcohols, or solutions of the nucleic acids in liquid or solid oil bases. The solutions may also contain buffers, diluents and other suitable additives. Pharmaceutically acceptable organic or inorganic excipients suitable for non-parenteral administration which do not deleteriously
10 react with nucleic acids can be used.

Suitable pharmaceutically acceptable excipients include, but are not limited to, water, salt solutions, alcohol, polyethylene glycols, gelatin, lactose, amylose, magnesium stearate, talc, silicic acid, viscous paraffin,
15 hydroxymethylcellulose, polyvinylpyrrolidone and the like.

Other Components

The compositions of the present invention may additionally contain other adjunct components conventionally found in pharmaceutical compositions, at
20 their art-established usage levels. Thus, for example, the compositions may contain additional, compatible, pharmaceutically-active materials such as, for example, antipruritics, astringents, local anesthetics or anti-inflammatory agents, or may contain additional
25 materials useful in physically formulating various dosage forms of the compositions of the present invention, such as dyes, flavoring agents, preservatives, antioxidants, opacifiers, thickening agents and stabilizers. However, such materials, when added, should not unduly interfere
30 with the biological activities of the components of the compositions of the present invention. The formulations can be sterilized and, if desired, mixed with auxiliary agents, e.g., lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic
35 pressure, buffers, colorings, flavorings and/or aromatic

-46-

substances and the like which do not deleteriously interact with the nucleic acid(s) of the formulation.

Aqueous suspensions may contain substances which increase the viscosity of the suspension including, for example, sodium carboxymethylcellulose, sorbitol and/or dextran. The suspension may also contain stabilizers.

Certain embodiments of the invention provide pharmaceutical compositions containing (a) one or more antisense compounds and (b) one or more other chemotherapeutic agents which function by a non-antisense mechanism. Examples of such chemotherapeutic agents include, but are not limited to, anticancer drugs such as daunorubicin, dactinomycin, doxorubicin, bleomycin, mitomycin, nitrogen mustard, chlorambucil, melphalan, cyclophosphamide, 6-mercaptopurine, 6-thioguanine, cytarabine (CA), 5-fluorouracil (5-FU), floxuridine (5-FUdR), methotrexate (MTX), colchicine, vincristine, vinblastine, etoposide, teniposide, cisplatin and diethylstilbestrol (DES). See, generally, *The Merck Manual of Diagnosis and Therapy*, 15th Ed., Berkow et al., eds., 1987, Rahway, N.J., pages 1206-1228). Anti-inflammatory drugs, including but not limited to nonsteroidal anti-inflammatory drugs and corticosteroids, and antiviral drugs, including but not limited to ribivirin, vidarabine, acyclovir and ganciclovir, may also be combined in compositions of the invention. See, generally, *The Merck Manual of Diagnosis and Therapy*, 15th Ed., Berkow et al., eds., 1987, Rahway, N.J., pages 2499-2506 and 46-49, respectively). Other non-antisense chemotherapeutic agents are also within the scope of this invention. Two or more combined compounds may be used together or sequentially.

In another related embodiment, compositions of the invention may contain one or more antisense compounds, particularly oligonucleotides, targeted to a first nucleic acid and one or more additional antisense compounds

-47-

targeted to a second nucleic acid target. Numerous examples of antisense compounds are known in the art. Two or more combined compounds may be used together or sequentially.

The formulation of therapeutic compositions and their subsequent administration is believed to be within the skill of those in the art. Dosing is dependent on severity and responsiveness of the disease state to be treated, with the course of treatment lasting from several days to several months, or until a cure is effected or a diminution of the disease state is achieved. Optimal dosing schedules can be calculated from measurements of drug accumulation in the body of the patient. Persons of ordinary skill can easily determine optimum dosages, dosing methodologies and repetition rates. Optimum dosages may vary depending on the relative potency of individual oligonucleotides, and can generally be estimated based on EC_{50} s found to be effective in in vitro and in vivo animal models. In general, dosage is from 0.01 ug to 100 g per kg of body weight, and may be given once or more daily, weekly, monthly or yearly, or even once every 2 to 20 years. Persons of ordinary skill in the art can easily estimate repetition rates for dosing based on measured residence times and concentrations of the drug in bodily fluids or tissues. Following successful treatment, it may be desirable to have the patient undergo maintenance therapy to prevent the recurrence of the disease state, wherein the oligonucleotide is administered in maintenance doses, ranging from 0.01 ug to 100 g per kg of body weight, once or more daily, to once every 20 years.

While the present invention has been described with specificity in accordance with certain of its preferred embodiments, the following examples serve only to illustrate the invention and are not intended to limit the same.

EXAMPLES**Example 1****Nucleoside Phosphoramidites for Oligonucleotide Synthesis****5 Deoxy and 2'-alkoxy amidites**

2'-Deoxy and 2'-methoxy beta-cyanoethyldiisopropyl phosphoramidites were purchased from commercial sources (e.g. Chemgenes, Needham MA or Glen Research, Inc. Sterling VA). Other 2'-O-alkoxy substituted nucleoside amidites are
10 prepared as described in U.S. Patent 5,506,351, herein incorporated by reference. For oligonucleotides synthesized using 2'-alkoxy amidites, the standard cycle for unmodified oligonucleotides was utilized, except the wait step after pulse delivery of tetrazole and base was
15 increased to 360 seconds.

Oligonucleotides containing 5-methyl-2'-deoxycytidine (5-Me-C) nucleotides were synthesized according to published methods [Sanghvi, et. al., *Nucleic Acids Research*, 1993, 21, 3197-3203] using commercially available
20 phosphoramidites (Glen Research, Sterling VA or ChemGenes, Needham MA).

2'-Fluoro amidites**2'-Fluorodeoxyadenosine amidites**

2'-fluoro oligonucleotides were synthesized as
25 described previously [Kawasaki, et. al., *J. Med. Chem.*, 1993, 36, 831-841] and United States patent 5,670,633, herein incorporated by reference. Briefly, the protected nucleoside N6-benzoyl-2'-deoxy-2'-fluoroadenosine was synthesized utilizing commercially available 9-beta-D-
30 arabinofuranosyladenine as starting material and by modifying literature procedures whereby the 2'-alpha-fluoro atom is introduced by a S_N2-displacement of a 2'-beta-trityl group. Thus N6-benzoyl-9-beta-D-arabinofuranosyladenine was selectively protected in moderate yield as the 3',5'-

-49-

ditetrahydropyranyl (THP) intermediate. Deprotection of the THP and N6-benzoyl groups was accomplished using standard methodologies and standard methods were used to obtain the 5'-dimethoxytrityl-(DMT) and 5'-DMT-3'-

5 phosphoramidite intermediates.

2'-Fluorodeoxyguanosine

The synthesis of 2'-deoxy-2'-fluoroguanosine was accomplished using tetraisopropylidisiloxanyl (TPDS) protected 9-beta-D-arabinofuranosylguanine as starting material, and conversion to the intermediate diisobutyryl-arabinofuranosylguanosine. Deprotection of the TPDS group was followed by protection of the hydroxyl group with THP to give diisobutyryl di-THP protected arabinofuranosylguanine. Selective O-deacylation and triflation was followed by treatment of the crude product with fluoride, then deprotection of the THP groups. Standard methodologies were used to obtain the 5'-DMT- and 5'-DMT-3'-phosphoramidites.

2'-Fluorouridine

Synthesis of 2'-deoxy-2'-fluorouridine was accomplished by the modification of a literature procedure in which 2,2'-anhydro-1-beta-D-arabinofuranosyluracil was treated with 70% hydrogen fluoride-pyridine. Standard procedures were used to obtain the 5'-DMT and 5'-DMT-3'-phosphoramidites.

2'-Fluorodeoxycytidine

2'-deoxy-2'-fluorocytidine was synthesized via amination of 2'-deoxy-2'-fluorouridine, followed by selective protection to give N4-benzoyl-2'-deoxy-2'-fluorocytidine. Standard procedures were used to obtain the 5'-DMT and 5'-DMT-3'-phosphoramidites.

2'-O-(2-Methoxyethyl) modified amidites

2'-O-Methoxyethyl-substituted nucleoside amidites are prepared as follows, or alternatively, as per the methods

-50-

of Martin, P., *Helvetica Chimica Acta*, 1995, 78, 486-504.

2,2'-Anhydro[1-(beta-D-arabinofuranosyl)-5-methyluridine]

5-Methyluridine (ribosylthymine, commercially

5 available through Yamasa, Choshi, Japan) (72.0 g, 0.279 M), diphenylcarbonate (90.0 g, 0.420 M) and sodium bicarbonate (2.0 g, 0.024 M) were added to DMF (300 mL). The mixture was heated to reflux, with stirring, allowing the evolved carbon dioxide gas to be released in a controlled manner. 10 After 1 hour, the slightly darkened solution was concentrated under reduced pressure. The resulting syrup was poured into diethylether (2.5 L), with stirring. The product formed a gum. The ether was decanted and the residue was dissolved in a minimum amount of methanol (ca. 15 400 mL). The solution was poured into fresh ether (2.5 L) to yield a stiff gum. The ether was decanted and the gum was dried in a vacuum oven (60°C at 1 mm Hg for 24 h) to give a solid that was crushed to a light tan powder (57 g, 85% crude yield). The NMR spectrum was consistent with the 20 structure, contaminated with phenol as its sodium salt (ca. 5%). The material was used as is for further reactions (or it can be purified further by column chromatography using a gradient of methanol in ethyl acetate (10-25%) to give a white solid, mp 222-4°C).

25 **2'-O-Methoxyethyl-5-methyluridine**

2,2'-Anhydro-5-methyluridine (195 g, 0.81 M), tris(2-methoxyethyl)borate (231 g, 0.98 M) and 2-methoxyethanol (1.2 L) were added to a 2 L stainless steel pressure vessel and placed in a pre-heated oil bath at 160°C. After heating 30 for 48 hours at 155-160°C, the vessel was opened and the solution evaporated to dryness and triturated with MeOH (200 mL). The residue was suspended in hot acetone (1 L). The insoluble salts were filtered, washed with acetone (150 mL) and the filtrate evaporated. The residue (280 g) was 35 dissolved in CH₃CN (600 mL) and evaporated. A silica gel

-51-

column (3 kg) was packed in CH_2Cl_2 /acetone/MeOH (20:5:3) containing 0.5% Et_3NH . The residue was dissolved in CH_2Cl_2 (250 mL) and adsorbed onto silica (150 g) prior to loading onto the column. The product was eluted with the packing solvent to give 160 g (63%) of product. Additional material was obtained by reworking impure fractions.

2'-O-Methoxyethyl-5'-O-dimethoxytrityl-5-methyluridine

2'-O-Methoxyethyl-5-methyluridine (160 g, 0.506 M) was co-evaporated with pyridine (250 mL) and the dried residue dissolved in pyridine (1.3 L). A first aliquot of dimethoxytrityl chloride (94.3 g, 0.278 M) was added and the mixture stirred at room temperature for one hour. A second aliquot of dimethoxytrityl chloride (94.3 g, 0.278 M) was added and the reaction stirred for an additional one hour. Methanol (170 mL) was then added to stop the reaction. HPLC showed the presence of approximately 70% product. The solvent was evaporated and triturated with CH_3CN (200 mL). The residue was dissolved in CHCl_3 (1.5 L) and extracted with 2x500 mL of saturated NaHCO_3 and 2x500 mL of saturated NaCl . The organic phase was dried over Na_2SO_4 , filtered and evaporated. 275 g of residue was obtained. The residue was purified on a 3.5 kg silica gel column, packed and eluted with EtOAc/hexane/acetone (5:5:1) containing 0.5% Et_3NH . The pure fractions were evaporated to give 164 g of product. Approximately 20 g additional was obtained from the impure fractions to give a total yield of 183 g (57%).

3'-O-Acetyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methyluridine

2'-O-Methoxyethyl-5'-O-dimethoxytrityl-5-methyluridine (106 g, 0.167 M), DMF/pyridine (750 mL of a 3:1 mixture prepared from 562 mL of DMF and 188 mL of pyridine) and acetic anhydride (24.38 mL, 0.258 M) were combined and stirred at room temperature for 24 hours. The reaction was monitored by TLC by first quenching the TLC sample with the

-52-

addition of MeOH. Upon completion of the reaction, as judged by TLC, MeOH (50 mL) was added and the mixture evaporated at 35°C. The residue was dissolved in CHCl₃ (800 mL) and extracted with 2x200 mL of saturated sodium bicarbonate and 2x200 mL of saturated NaCl. The water layers were back extracted with 200 mL of CHCl₃. The combined organics were dried with sodium sulfate and evaporated to give 122 g of residue (approx. 90% product). The residue was purified on a 3.5 kg silica gel column and eluted using EtOAc/hexane(4:1). Pure product fractions were evaporated to yield 96 g (84%). An additional 1.5 g was recovered from later fractions.

3'-O-Acetyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methyl-4-triazoleuridine

A first solution was prepared by dissolving 3'-O-acetyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methyluridine (96 g, 0.144 M) in CH₃CN (700 mL) and set aside. Triethylamine (189 mL, 1.44 M) was added to a solution of triazole (90 g, 1.3 M) in CH₃CN (1 L), cooled to -5°C and stirred for 0.5 h using an overhead stirrer. POCl₃ was added dropwise, over a 30 minute period, to the stirred solution maintained at 0-10°C, and the resulting mixture stirred for an additional 2 hours. The first solution was added dropwise, over a 45 minute period, to the latter solution. The resulting reaction mixture was stored overnight in a cold room. Salts were filtered from the reaction mixture and the solution was evaporated. The residue was dissolved in EtOAc (1 L) and the insoluble solids were removed by filtration. The filtrate was washed with 1x300 mL of NaHCO₃ and 2x300 mL of saturated NaCl, dried over sodium sulfate and evaporated. The residue was triturated with EtOAc to give the title compound.

2'-O-Methoxyethyl-5'-O-dimethoxytrityl-5-methylcytidine

A solution of 3'-O-acetyl-2'-O-methoxyethyl-5'-O-

-53-

dimethoxytrityl-5-methyl-4-triazoleuridine (103 g, 0.141 M) in dioxane (500 mL) and NH_4OH (30 mL) was stirred at room temperature for 2 hours. The dioxane solution was evaporated and the residue azeotrope with MeOH (2x200 mL).

5 The residue was dissolved in MeOH (300 mL) and transferred to a 2 liter stainless steel pressure vessel. MeOH (400 mL) saturated with NH_3 gas was added and the vessel heated to 100°C for 2 hours (TLC showed complete conversion). The vessel contents were evaporated to dryness and the residue
10 was dissolved in EtOAc (500 mL) and washed once with saturated NaCl (200 mL). The organics were dried over sodium sulfate and the solvent was evaporated to give 85 g (95%) of the title compound.

N4-Benzoyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methylcytidine

15 2'-O-Methoxyethyl-5'-O-dimethoxytrityl-5-methylcytidine (85 g, 0.134 M) was dissolved in DMF (800 mL) and benzoic anhydride (37.2 g, 0.165 M) was added with stirring. After stirring for 3 hours, TLC showed the
20 reaction to be approximately 95% complete. The solvent was evaporated and the residue azeotrope with MeOH (200 mL). The residue was dissolved in CHCl_3 (700 mL) and extracted with saturated NaHCO_3 (2x300 mL) and saturated NaCl (2x300 mL), dried over MgSO_4 and evaporated to give a residue (96
25 g). The residue was chromatographed on a 1.5 kg silica column using EtOAc/hexane (1:1) containing 0.5% Et_3NH as the eluting solvent. The pure product fractions were evaporated to give 90 g (90%) of the title compound.

N4-Benzoyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methylcytidine-3'-amidite

30 N4-Benzoyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methylcytidine (74 g, 0.10 M) was dissolved in CH_2Cl_2 (1 L). Tetrazole diisopropylamine (7.1 g) and 2-cyanoethoxy-tetra-(isopropyl)phosphite (40.5 mL, 0.123 M) were added with
35 stirring, under a nitrogen atmosphere. The resulting

-54-

204420 9492650

mixture was stirred for 20 hours at room temperature (TLC showed the reaction to be 95% complete). The reaction mixture was extracted with saturated NaHCO_3 (1x300 mL) and saturated NaCl (3x300 mL). The aqueous washes were back-extracted with CH_2Cl_2 (300 mL), and the extracts were combined, dried over MgSO_4 and concentrated. The residue obtained was chromatographed on a 1.5 kg silica column using EtOAc /hexane (3:1) as the eluting solvent. The pure fractions were combined to give 90.6 g (87%) of the title compound.

2'-O-(Aminooxyethyl) nucleoside amidites and 2'-O-(dimethylaminooxyethyl) nucleoside amidites

2'-(Dimethylaminooxyethoxy) nucleoside amidites

2'-(Dimethylaminooxyethoxy) nucleoside amidites [also known in the art as 2'-O-(dimethylaminooxyethyl) nucleoside amidites] are prepared as described in the following paragraphs. Adenosine, cytidine and guanosine nucleoside amidites are prepared similarly to the thymidine (5-methyluridine) except the exocyclic amines are protected with a benzoyl moiety in the case of adenosine and cytidine and with isobutyryl in the case of guanosine.

5'-O-tert-Butyldiphenylsilyl-O²-2'-anhydro-5-methyluridine

O²-2'-anhydro-5-methyluridine (Pro. Bio. Sint., Varese, Italy, 100.0g, 0.416 mmol), dimethylaminopyridine (0.66g, 0.013eq, 0.0054mmol) were dissolved in dry pyridine (500 ml) at ambient temperature under an argon atmosphere and with mechanical stirring. *tert*-Butyldiphenylchlorosilane (125.8g, 119.0mL, 1.1eq, 0.458mmol) was added in one portion. The reaction was stirred for 16 h at ambient temperature. TLC (R_f 0.22, ethyl acetate) indicated a complete reaction. The solution was concentrated under reduced pressure to a thick oil. This was partitioned between dichloromethane (1 L) and saturated sodium

-55-

bicarbonate (2x1 L) and brine (1 L). The organic layer was dried over sodium sulfate and concentrated under reduced pressure to a thick oil. The oil was dissolved in a 1:1 mixture of ethyl acetate and ethyl ether (600mL) and the solution was cooled to -10°C. The resulting crystalline product was collected by filtration, washed with ethyl ether (3x200 mL) and dried (40°C, 1mm Hg, 24 h) to 149g (74.8%) of white solid. TLC and NMR were consistent with pure product.

10 **5'-O-tert-Butyldiphenylsilyl-2'-O-(2-hydroxyethyl)-5-methyluridine**

In a 2 L stainless steel, unstirred pressure reactor was added borane in tetrahydrofuran (1.0 M, 2.0 eq, 622 mL). In the fume hood and with manual stirring, ethylene glycol (350 mL, excess) was added cautiously at first until the evolution of hydrogen gas subsided. 5'-O-tert-Butyldiphenylsilyl-O²-2'-anhydro-5-methyluridine (149 g, 0.311 mol) and sodium bicarbonate (0.074 g, 0.003 eq) were added with manual stirring. The reactor was sealed and heated in an oil bath until an internal temperature of 160 °C was reached and then maintained for 16 h (pressure < 100 psig). The reaction vessel was cooled to ambient and opened. TLC (R_f 0.67 for desired product and R_f 0.82 for ara-T side product, ethyl acetate) indicated about 70% conversion to the product. In order to avoid additional side product formation, the reaction was stopped, concentrated under reduced pressure (10 to 1mm Hg) in a warm water bath (40-100°C) with the more extreme conditions used to remove the ethylene glycol. [Alternatively, once the low boiling solvent is gone, the remaining solution can be partitioned between ethyl acetate and water. The product will be in the organic phase.] The residue was purified by column chromatography (2kg silica gel, ethyl acetate-hexanes gradient 1:1 to 4:1). The appropriate fractions were combined, stripped and dried to product as a

-56-

white crisp foam (84g, 50%), contaminated starting material (17.4g) and pure reusable starting material 20g. The yield based on starting material less pure recovered starting material was 58%. TLC and NMR were consistent with 99%

5 pure product.

2'-O-([2-phthalimidoxy)ethyl]-5'-t-butyldiphenylsilyl-5-methyluridine

5'-O-tert-Butyldiphenylsilyl-2'-O-(2-hydroxyethyl)-5-methyluridine (20g, 36.98mmol) was mixed with
10 triphenylphosphine (11.63g, 44.36mmol) and N-hydroxyphthalimide (7.24g, 44.36mmol). It was then dried over P₂O₅ under high vacuum for two days at 40°C. The reaction mixture was flushed with argon and dry THF (369.8mL, Aldrich, sure seal bottle) was added to get a
15 clear solution. Diethyl-azodicarboxylate (6.98mL, 44.36mmol) was added dropwise to the reaction mixture. The rate of addition is maintained such that resulting deep red coloration is just discharged before adding the next drop. After the addition was complete, the reaction was stirred
20 for 4 hrs. By that time TLC showed the completion of the reaction (ethylacetate:hexane, 60:40). The solvent was evaporated in vacuum. Residue obtained was placed on a flash column and eluted with ethyl acetate:hexane (60:40), to get 2'-O-([2-phthalimidoxy)ethyl]-5'-t-
25 butyldiphenylsilyl-5-methyluridine as white foam (21.819 g, 86%).

5'-O-tert-butyldiphenylsilyl-2'-O-[(2-formadoximinooxy)ethyl]-5-methyluridine

2'-O-([2-phthalimidoxy)ethyl]-5'-t-butyldiphenylsilyl-
30 5-methyluridine (3.1g, 4.5mmol) was dissolved in dry CH₂Cl₂ (4.5mL) and methylhydrazine (300mL, 4.64mmol) was added dropwise at -10°C to 0°C. After 1 h the mixture was filtered, the filtrate was washed with ice cold CH₂Cl₂ and the combined organic phase was washed with water, brine and

-57-

dried over anhydrous Na_2SO_4 . The solution was concentrated to get 2'-O-(aminooxyethyl) thymidine, which was then dissolved in MeOH (67.5mL). To this formaldehyde (20% aqueous solution, w/w, 1.1 eq.) was added and the resulting mixture was stirred for 1 h. Solvent was removed under vacuum; residue chromatographed to get 5'-O-tert-butylidiphenylsilyl-2'-O-[(2-formadoximinooxy) ethyl]-5-methyluridine as white foam (1.95 g, 78%).

5'-O-tert-Butylidiphenylsilyl-2'-O-[N,N-dimethylaminooxyethyl]-5-methyluridine

5'-O-tert-butylidiphenylsilyl-2'-O-[(2-formadoximinooxy)ethyl]-5-methyluridine (1.77g, 3.12mmol) was dissolved in a solution of 1M pyridinium p-toluenesulfonate (PPTS) in dry MeOH (30.6mL). Sodium cyanoborohydride (0.39g, 6.13mmol) was added to this solution at 10°C under inert atmosphere. The reaction mixture was stirred for 10 minutes at 10°C. After that the reaction vessel was removed from the ice bath and stirred at room temperature for 2 h, the reaction monitored by TLC (5% MeOH in CH_2Cl_2). Aqueous NaHCO_3 solution (5%, 10mL) was added and extracted with ethyl acetate (2x20mL). Ethyl acetate phase was dried over anhydrous Na_2SO_4 , evaporated to dryness. Residue was dissolved in a solution of 1M PPTS in MeOH (30.6mL). Formaldehyde (20% w/w, 30mL, 3.37mmol) was added and the reaction mixture was stirred at room temperature for 10 minutes. Reaction mixture cooled to 10°C in an ice bath, sodium cyanoborohydride (0.39g, 6.13mmol) was added and reaction mixture stirred at 10°C for 10 minutes. After 10 minutes, the reaction mixture was removed from the ice bath and stirred at room temperature for 2 hrs. To the reaction mixture 5% NaHCO_3 (25mL) solution was added and extracted with ethyl acetate (2x25mL). Ethyl acetate layer was dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue obtained was

-58-

purified by flash column chromatography and eluted with 5% MeOH in CH₂Cl₂ to get 5'-O-tert-butyldiphenylsilyl-2'-O-[N,N-dimethylaminooxyethyl]-5-methyluridine as a white foam (14.6g, 80%).

5 **2'-O-(dimethylaminooxyethyl)-5-methyluridine**

Triethylamine trihydrofluoride (3.91mL, 24.0mmol) was dissolved in dry THF and triethylamine (1.67mL, 12mmol, dry, kept over KOH). This mixture of triethylamine-2HF was then added to 5'-O-tert-butyldiphenylsilyl-2'-O-[N,N-
10 dimethylaminooxyethyl]-5-methyluridine (1.40g, 2.4mmol) and stirred at room temperature for 24 hrs. Reaction was monitored by TLC (5% MeOH in CH₂Cl₂). Solvent was removed under vacuum and the residue placed on a flash column and eluted with 10% MeOH in CH₂Cl₂ to get 2'-O-
15 (dimethylaminooxyethyl)-5-methyluridine (766mg, 92.5%).

5'-O-DMT-2'-O-(dimethylaminooxyethyl)-5-methyluridine

2'-O-(dimethylaminooxyethyl)-5-methyluridine (750mg, 2.17mmol) was dried over P₂O₅ under high vacuum overnight at 40°C. It was then co-evaporated with anhydrous pyridine
20 (20mL). The residue obtained was dissolved in pyridine (11mL) under argon atmosphere. 4-dimethylaminopyridine (26.5mg, 2.60mmol), 4,4'-dimethoxytrityl chloride (880mg, 2.60mmol) was added to the mixture and the reaction mixture was stirred at room temperature until all of the starting
25 material disappeared. Pyridine was removed under vacuum and the residue chromatographed and eluted with 10% MeOH in CH₂Cl₂ (containing a few drops of pyridine) to get 5'-O-DMT-2'-O-(dimethylamino-oxyethyl)-5-methyluridine (1.13g, 80%).

30 **5'-O-DMT-2'-O-(2-N,N-dimethylaminooxyethyl)-5-methyluridine-3'-[(2-cyanoethyl)-N,N-diisopropylphosphoramidite]**

5'-O-DMT-2'-O-(dimethylaminooxyethyl)-5-methyluridine (1.08g, 1.67mmol) was co-evaporated with toluene (20mL). To the residue N,N-diisopropylamine tetrazonide (0.29g,

-59-

1.67mmol) was added and dried over P_2O_5 under high vacuum overnight at $40^\circ C$. Then the reaction mixture was dissolved in anhydrous acetonitrile (8.4mL) and 2-cyanoethyl-N,N,N',N'-tetraisopropylphosphoramidite (2.12mL, 6.08mmol) was added. The reaction mixture was stirred at ambient temperature for 4 hrs under inert atmosphere. The progress of the reaction was monitored by TLC (hexane:ethyl acetate 1:1). The solvent was evaporated, then the residue was dissolved in ethyl acetate (70mL) and washed with 5% aqueous $NaHCO_3$ (40mL). Ethyl acetate layer was dried over anhydrous Na_2SO_4 and concentrated. Residue obtained was chromatographed (ethyl acetate as eluent) to get 5'-O-DMT-2'-O-(2-N,N-dimethylaminoxyethyl)-5-methyluridine-3'-[(2-cyanoethyl)-N,N-diisopropylphosphoramidite] as a foam (1.04g, 74.9%).

2'-(Aminooxyethoxy) nucleoside amidites

2'-(Aminooxyethoxy) nucleoside amidites [also known in the art as 2'-O-(aminooxyethyl) nucleoside amidites] are prepared as described in the following paragraphs.

Adenosine, cytidine and thymidine nucleoside amidites are prepared similarly.

N2-isobutyryl-6-O-diphenylcarbamoyl-2'-O-(2-ethylacetyl)-5'-O-(4,4'-dimethoxytrityl)guanosine-3'-[(2-cyanoethyl)-N,N-diisopropylphosphoramidite]

The 2'-O-aminooxyethyl guanosine analog may be obtained by selective 2'-O-alkylation of diaminopurine riboside. Multigram quantities of diaminopurine riboside may be purchased from Schering AG (Berlin) to provide 2'-O-(2-ethylacetyl) diaminopurine riboside along with a minor amount of the 3'-O-isomer. 2'-O-(2-ethylacetyl) diaminopurine riboside may be resolved and converted to 2'-O-(2-ethylacetyl)guanosine by treatment with adenosine deaminase. (McGee, D. P. C., Cook, P. D., Guinosso, C. J., WO 94/02501 A1 940203.) Standard protection procedures should afford 2'-O-(2-ethylacetyl)-5'-O-(4,4'-

-60-

dimethoxytrityl)guanosine and 2-N-isobutyryl-6-O-diphenylcarbamoyl-2'-O-(2-ethylacetyl)-5'-O-(4,4'-dimethoxytrityl)guanosine which may be reduced to provide 2-N-isobutyryl-6-O-diphenylcarbamoyl-2'-O-(2-ethylacetyl)-5'-O-(4,4'-dimethoxytrityl)guanosine. As before the hydroxyl group may be displaced by N-hydroxyphthalimide via a Mitsunobu reaction, and the protected nucleoside may phosphitylated as usual to yield 2-N-isobutyryl-6-O-diphenylcarbamoyl-2'-O-(2-ethylacetyl)-5'-O-(4,4'-dimethoxytrityl)guanosine-3'-[(2-cyanoethyl)-N,N-diisopropylphosphoramidite].

Example 2

Oligonucleotide synthesis

Unsubstituted and substituted phosphodiester (P=O) oligonucleotides are synthesized on an automated DNA synthesizer (Applied Biosystems model 380B) using standard phosphoramidite chemistry with oxidation by iodine.

Phosphorothioates (P=S) are synthesized as for the phosphodiester oligonucleotides except the standard oxidation bottle was replaced by 0.2 M solution of 3H-1,2-benzodithiole-3-one 1,1-dioxide in acetonitrile for the stepwise thiation of the phosphite linkages. The thiation wait step was increased to 68 sec and was followed by the capping step. After cleavage from the CPG column and deblocking in concentrated ammonium hydroxide at 55°C (18 h), the oligonucleotides were purified by precipitating twice with 2.5 volumes of ethanol from a 0.5 M NaCl solution. Phosphate oligonucleotides are prepared as described in U.S. Patent 5,508,270, herein incorporated by reference.

Alkyl phosphonate oligonucleotides are prepared as described in U.S. Patent 4,469,863, herein incorporated by reference.

3'-Deoxy-3'-methylene phosphonate oligonucleotides are prepared as described in U.S. Patents 5,610,289 or

-61-

5,625,050, herein incorporated by reference.

Phosphoramidite oligonucleotides are prepared as described in U.S. Patent, 5,256,775 or U.S. Patent 5,366,878, herein incorporated by reference.

5 Alkylphosphonothioate oligonucleotides are prepared as described in published PCT applications PCT/US94/00902 and PCT/US93/06976 (published as WO 94/17093 and WO 94/02499, respectively), herein incorporated by reference.

10 3'-Deoxy-3'-amino phosphoramidate oligonucleotides are prepared as described in U.S. Patent 5,476,925, herein incorporated by reference.

Phosphotriester oligonucleotides are prepared as described in U.S. Patent 5,023,243, herein incorporated by reference.

15 Borano phosphate oligonucleotides are prepared as described in U.S. Patents 5,130,302 and 5,177,198, both herein incorporated by reference.

Example 3

Oligonucleoside Synthesis

20 Methylenemethylimino linked oligonucleosides, also identified as MMI linked oligonucleosides, methylenedi-methylhydrazo linked oligonucleosides, also identified as MDH linked oligonucleosides, and methylenecarbonylamino linked oligonucleosides, also identified as amide-3 linked
25 oligonucleosides, and methyleneaminocarbonyl linked oligonucleosides, also identified as amide-4 linked oligonucleosides, as well as mixed backbone compounds having, for instance, alternating MMI and P=O or P=S linkages are prepared as described in U.S. Patents 5,378,825, 5,386,023,
30 5,489,677, 5,602,240 and 5,610,289, all of which are herein incorporated by reference.

Formacetal and thioformacetal linked oligonucleosides are prepared as described in U.S. Patents 5,264,562 and 5,264,564, herein incorporated by reference.

-62-

Ethylene oxide linked oligonucleosides are prepared as described in U.S. Patent 5,223,618, herein incorporated by reference.

Example 4**5 PNA Synthesis**

Peptide nucleic acids (PNAs) are prepared in accordance with any of the various procedures referred to in Peptide Nucleic Acids (PNA): Synthesis, Properties and Potential Applications, *Bioorganic & Medicinal Chemistry*,
10 1996, 4, 5-23. They may also be prepared in accordance with U.S. Patents 5,539,082, 5,700,922, and 5,719,262, herein incorporated by reference.

Example 5**Synthesis of Chimeric Oligonucleotides**

15 Chimeric oligonucleotides, oligonucleosides or mixed oligonucleotides/oligonucleosides of the invention can be of several different types. These include a first type wherein the "gap" segment of linked nucleosides is positioned between 5' and 3' "wing" segments of linked
20 nucleosides and a second "open end" type wherein the "gap" segment is located at either the 3' or the 5' terminus of the oligomeric compound. Oligonucleotides of the first type are also known in the art as "gapmers" or gapped oligonucleotides. Oligonucleotides of the second type are
25 also known in the art as "hemimers" or "wingmers".

[2'-O-Me]--[2'-deoxy]--[2'-O-Me] Chimeric**Phosphorothioate Oligonucleotides**

Chimeric oligonucleotides having 2'-O-alkyl phosphorothioate and 2'-deoxy phosphorothioate oligo-
30 nucleotide segments are synthesized using an Applied Biosystems automated DNA synthesizer Model 380B, as above. Oligonucleotides are synthesized using the automated synthesizer and 2'-deoxy-5'-dimethoxytrityl-3'-O-phosphor-
amidite for the DNA portion and 5'-dimethoxytrityl-2'-O-

-63-

methyl-3'-O-phosphoramidite for 5' and 3' wings. The
 standard synthesis cycle is modified by increasing the wait
 step after the delivery of tetrazole and base to 600 s
 repeated four times for RNA and twice for 2'-O-methyl. The
 5 fully protected oligonucleotide is cleaved from the support
 and the phosphate group is deprotected in 3:1
 ammonia/ethanol at room temperature overnight then
 lyophilized to dryness. Treatment in methanolic ammonia
 for 24 hrs at room temperature is then done to deprotect
 10 all bases and sample was again lyophilized to dryness. The
 pellet is resuspended in 1M TBAF in THF for 24 hrs at room
 temperature to deprotect the 2' positions. The reaction is
 then quenched with 1M TEAA and the sample is then reduced
 to 1/2 volume by rotovac before being desalted on a G25
 15 size exclusion column. The oligo recovered is then
 analyzed spectrophotometrically for yield and for purity by
 capillary electrophoresis and by mass spectrometry.

[2'-O-(2-Methoxyethyl)]--[2'-deoxy]--[2'-O-
 (Methoxyethyl)] Chimeric Phosphorothioate

20 Oligonucleotides

[2'-O-(2-methoxyethyl)]--[2'-deoxy]--[2'-O-(methoxy-
 ethyl)] chimeric phosphorothioate oligonucleotides were
 prepared as per the procedure above for the 2'-O-methyl
 chimeric oligonucleotide, with the substitution of 2'-O-
 25 (methoxyethyl) amidites for the 2'-O-methyl amidites.

[2'-O-(2-Methoxyethyl)Phosphodiester]--[2'-deoxy
 Phosphorothioate]--[2'-O-(2-Methoxyethyl)
 Phosphodiester] Chimeric Oligonucleotides

[2'-O-(2-methoxyethyl phosphodiester)]--[2'-deoxy phos-
 30 phorothioate]--[2'-O-(methoxyethyl) phosphodiester]
 chimeric oligonucleotides are prepared as per the above
 procedure for the 2'-O-methyl chimeric oligonucleotide with
 the substitution of 2'-O-(methoxyethyl) amidites for the
 2'-O-methyl amidites, oxidization with iodine to generate

-64-

the phosphodiester internucleotide linkages within the wing portions of the chimeric structures and sulfurization utilizing 3,4-dihydro-2H-benzothio-1,2-dioxole-3-one 1,1-dioxide (Beaucage Reagent) to generate the phosphorothioate internucleotide linkages for the center gap.

Other chimeric oligonucleotides, chimeric oligonucleosides and mixed chimeric oligonucleotides/oligonucleosides are synthesized according to United States patent 5,623,065, herein incorporated by reference.

10 Example 6

Oligonucleotide Isolation

After cleavage from the controlled pore glass column (Applied Biosystems) and deblocking in concentrated ammonium hydroxide at 55°C for 18 hours, the oligonucleotides or oligonucleosides are purified by precipitation twice out of 0.5 M NaCl with 2.5 volumes ethanol. Synthesized oligonucleotides were analyzed by polyacrylamide gel electrophoresis on denaturing gels and judged to be at least 85% full length material. The relative amounts of phosphorothioate and phosphodiester linkages obtained in synthesis were periodically checked by ³¹P nuclear magnetic resonance spectroscopy, and for some studies oligonucleotides were purified by HPLC, as described by Chiang et al., *J. Biol. Chem.* 1991, 266, 18162-18171. Results obtained with HPLC-purified material were similar to those obtained with non-HPLC purified material.

Example 7

Oligonucleotide Synthesis - 96 Well Plate Format

Oligonucleotides were synthesized via solid phase P(III) phosphoramidite chemistry on an automated synthesizer capable of assembling 96 sequences simultaneously in a standard 96 well format. Phosphodiester internucleotide linkages were afforded by

-65-

oxidation with aqueous iodine. Phosphorothioate internucleotide linkages were generated by sulfurization utilizing 3,4-dihydro-2H-benzothiole-3-one 1,1-dioxide (Beaucage Reagent) in anhydrous acetonitrile. Standard base-

5 protected beta-cyanoethyl-diisopropyl phosphoramidites were purchased from commercial vendors (e.g. PE-Applied Biosystems, Foster City, CA, or Pharmacia, Piscataway, NJ). Non-standard nucleosides are synthesized as per known literature or patented methods. They are utilized as base
10 protected beta-cyanoethyl-diisopropyl phosphoramidites.

Oligonucleotides were cleaved from support and deprotected with concentrated NH_4OH at elevated temperature ($55-60^\circ\text{C}$) for 12-16 hours and the released product then dried in vacuo. The dried product was then re-suspended in
15 sterile water to afford a master plate from which all analytical and test plate samples are then diluted utilizing robotic pipettors.

Example 8

Oligonucleotide Analysis - 96 Well Plate Format

20 The concentration of oligonucleotide in each well was assessed by dilution of samples and UV absorption spectroscopy. The full-length integrity of the individual products was evaluated by capillary electrophoresis (CE) in either the 96 well format (Beckman P/ACE™ MDQ) or, for
25 individually prepared samples, on a commercial CE apparatus (e.g., Beckman P/ACE™ 5000, ABI 270). Base and backbone composition was confirmed by mass analysis of the compounds utilizing electrospray-mass spectroscopy. All assay test
30 plates were diluted from the master plate using single and multi-channel robotic pipettors. Plates were judged to be acceptable if at least 85% of the compounds on the plate were at least 85% full length.

Example 9

Cell culture and oligonucleotide treatment

35 The effect of antisense compounds on target nucleic

-66-

acid expression can be tested in any of a variety of cell types provided that the target nucleic acid is present at measurable levels. This can be routinely determined using, for example, PCR or Northern blot analysis. The following
5 four cell types are provided for illustrative purposes, but other cell types can be routinely used.

T-24 cells:

The transitional cell bladder carcinoma cell line T-24 was obtained from the American Type Culture Collection
10 (ATCC) (Manassas, VA). T-24 cells were routinely cultured in complete McCoy's 5A basal media (Gibco/Life Technologies, Gaithersburg, MD) supplemented with 10% fetal calf serum (Gibco/Life Technologies, Gaithersburg, MD), penicillin 100 units per mL, and streptomycin 100
15 micrograms per mL (Gibco/Life Technologies, Gaithersburg, MD). Cells were routinely passaged by trypsinization and dilution when they reached 90% confluence. Cells were seeded into 96-well plates (Falcon-Primaria #3872) at a density of 7000 cells/well for use in RT-PCR analysis.

20 For Northern blotting or other analysis, cells may be seeded onto 100 mm or other standard tissue culture plates and treated similarly, using appropriate volumes of medium and oligonucleotide.

A549 cells:

25 The human lung carcinoma cell line A549 was obtained from the American Type Culture Collection (ATCC) (Manassas, VA). A549 cells were routinely cultured in DMEM basal media (Gibco/Life Technologies, Gaithersburg, MD) supplemented with 10% fetal calf serum (Gibco/Life
30 Technologies, Gaithersburg, MD), penicillin 100 units per mL, and streptomycin 100 micrograms per mL (Gibco/Life Technologies, Gaithersburg, MD). Cells were routinely passaged by trypsinization and dilution when they reached 90% confluence.

-67-

NHDF cells:

Human neonatal dermal fibroblast (NHDF) were obtained from the Clonetics Corporation (Walkersville MD). NHDFs were routinely maintained in Fibroblast Growth Medium

- 5 (Clonetics Corporation, Walkersville MD) supplemented as recommended by the supplier. Cells were maintained for up to 10 passages as recommended by the supplier.

HEK cells:

- Human embryonic keratinocytes (HEK) were obtained from
10 the Clonetics Corporation (Walkersville MD). HEKs were routinely maintained in Keratinocyte Growth Medium (Clonetics Corporation, Walkersville MD) formulated as recommended by the supplier. Cells were routinely maintained for up to 10 passages as recommended by the
15 supplier.

Treatment with antisense compounds:

- When cells reached 80% confluency, they were treated with oligonucleotide. For cells grown in 96-well plates, wells were washed once with 200 μ L OPTI-MEM™-1 reduced-
20 serum medium (Gibco BRL) and then treated with 130 μ L of OPTI-MEM™-1 containing 3.75 μ g/mL LIPOFECTIN™ (Gibco BRL) and the desired oligonucleotide at a final concentration of 150 nM. After 4 hours of treatment, the medium was replaced with fresh medium. Cells were harvested 16 hours
25 after oligonucleotide treatment.

Example 10**Analysis of oligonucleotide inhibition of SRA expression**

- Antisense modulation of SRA or SRA expression can be assayed in a variety of ways known in the art. For
30 example, SRA mRNA levels can be quantitated by, e.g., Northern blot analysis, competitive polymerase chain reaction (PCR), or real-time PCR (RT-PCR). Real-time quantitative PCR is presently preferred. RNA analysis can be performed on total cellular RNA or poly(A)+ mRNA.
35 Methods of RNA isolation are taught in, for example,

-68-

Ausubel, F.M. et al., *Current Protocols in Molecular Biology*, Volume 1, pp. 4.1.1-4.2.9 and 4.5.1-4.5.3, John Wiley & Sons, Inc., 1993. Northern blot analysis is routine in the art and is taught in, for example, Ausubel, F.M. et al., *Current Protocols in Molecular Biology*, Volume 1, pp. 4.2.1-4.2.9, John Wiley & Sons, Inc., 1996. Real-time quantitative (PCR) can be conveniently accomplished using the commercially available ABI PRISM™ 7700 Sequence Detection System, available from PE-Applied Biosystems, Foster City, CA and used according to manufacturer's instructions. Other methods of PCR are also known in the art.

It is presently believed that SRA is not translated to a protein. However, if appropriate, protein levels can be quantitated in a variety of ways well known in the art, such as immunoprecipitation, Western blot analysis (immunoblotting), ELISA or fluorescence-activated cell sorting (FACS). Methods for preparation of polyclonal antisera are taught in, for example, Ausubel, F.M. et al., *Current Protocols in Molecular Biology*, Volume 2, pp. 11.12.1-11.12.9, John Wiley & Sons, Inc., 1997. Preparation of monoclonal antibodies is taught in, for example, Ausubel, F.M. et al., *Current Protocols in Molecular Biology*, Volume 2, pp. 11.4.1-11.11.5, John Wiley & Sons, Inc., 1997.

Immunoprecipitation methods are standard in the art and can be found at, for example, Ausubel, F.M. et al., *Current Protocols in Molecular Biology*, Volume 2, pp. 10.16.1-10.16.11, John Wiley & Sons, Inc., 1998. Western blot (immunoblot) analysis is standard in the art and can be found at, for example, Ausubel, F.M. et al., *Current Protocols in Molecular Biology*, Volume 2, pp. 10.8.1-10.8.21, John Wiley & Sons, Inc., 1997. Enzyme-linked immunosorbent assays (ELISA) are standard in the art and

-69-

can be found at, for example, Ausubel, F.M. et al., *Current Protocols in Molecular Biology*, Volume 2, pp. 11.2.1-11.2.22, John Wiley & Sons, Inc., 1991.

Example 11**5 Poly(A)+ mRNA isolation**

Poly(A)+ mRNA was isolated according to Miura et al., *Clin. Chem.*, 1996, 42, 1758-1764. Other methods for poly(A)+ mRNA isolation are taught in, for example, Ausubel, F.M. et al., *Current Protocols in Molecular*
10 *Biology*, Volume 1, pp. 4.5.1-4.5.3, John Wiley & Sons, Inc., 1993. Briefly, for cells grown on 96-well plates, growth medium was removed from the cells and each well was washed with 200 μ L cold PBS. 60 μ L lysis buffer (10 mM Tris-HCl, pH 7.6, 1 mM EDTA, 0.5 M NaCl, 0.5% NP-40, 20 mM
15 vanadyl-ribonucleoside complex) was added to each well, the plate was gently agitated and then incubated at room temperature for five minutes. 55 μ L of lysate was transferred to Oligo d(T) coated 96-well plates (AGCT Inc., Irvine CA). Plates were incubated for 60 minutes at room
20 temperature, washed 3 times with 200 μ L of wash buffer (10 mM Tris-HCl pH 7.6, 1 mM EDTA, 0.3 M NaCl). After the final wash, the plate was blotted on paper towels to remove excess wash buffer and then air-dried for 5 minutes. 60 μ L of elution buffer (5 mM Tris-HCl pH 7.6), preheated to 70°C
25 was added to each well, the plate was incubated on a 90°C hot plate for 5 minutes, and the eluate was then transferred to a fresh 96-well plate.

Cells grown on 100 mm or other standard plates may be treated similarly, using appropriate volumes of all
30 solutions.

Example 12**Total RNA Isolation**

Total mRNA was isolated using an RNEASY 96™ kit and buffers purchased from Qiagen Inc. (Valencia CA) following

-70-

the manufacturer's recommended procedures. Briefly, for cells grown on 96-well plates, growth medium was removed from the cells and each well was washed with 200 μ L cold PBS. 100 μ L Buffer RLT was added to each well and the plate vigorously agitated for 20 seconds. 100 μ L of 70% ethanol was then added to each well and the contents mixed by pipetting three times up and down. The samples were then transferred to the RNEASY 96™ well plate attached to a QIAVAC™ manifold fitted with a waste collection tray and attached to a vacuum source. Vacuum was applied for 15 seconds. 1 mL of Buffer RW1 was added to each well of the RNEASY 96™ plate and the vacuum again applied for 15 seconds. 1 mL of Buffer RPE was then added to each well of the RNEASY 96™ plate and the vacuum applied for a period of 15 seconds. The Buffer RPE wash was then repeated and the vacuum was applied for an additional 10 minutes. The plate was then removed from the QIAVAC™ manifold and blotted dry on paper towels. The plate was then re-attached to the QIAVAC™ manifold fitted with a collection tube rack containing 1.2 mL collection tubes. RNA was then eluted by pipetting 60 μ L water into each well, incubating 1 minute, and then applying the vacuum for 30 seconds. The elution step was repeated with an additional 60 μ L water.

Example 13**25 Real-time Quantitative PCR Analysis of SRA mRNA Levels**

Quantitation of SRA mRNA levels was determined by real-time quantitative PCR using the ABI PRISM™ 7700 Sequence Detection System (PE-Applied Biosystems, Foster City, CA) according to manufacturer's instructions. This is a closed-tube, non-gel-based, fluorescence detection system which allows high-throughput quantitation of polymerase chain reaction (PCR) products in real-time. As opposed to standard PCR, in which amplification products are quantitated after the PCR is completed, products in real-time quantitative PCR are quantitated as they

-71-

accumulate. This is accomplished by including in the PCR reaction an oligonucleotide probe that anneals specifically between the forward and reverse PCR primers, and contains two fluorescent dyes. A reporter dye (e.g., JOE or FAM, obtained from either Operon Technologies Inc., Alameda, CA or PE-Applied Biosystems, Foster City, CA) is attached to the 5' end of the probe and a quencher dye (e.g., TAMRA, obtained from either Operon Technologies Inc., Alameda, CA or PE-Applied Biosystems, Foster City, CA) is attached to the 3' end of the probe. When the probe and dyes are intact, reporter dye emission is quenched by the proximity of the 3' quencher dye. During amplification, annealing of the probe to the target sequence creates a substrate that can be cleaved by the 5'-exonuclease activity of Taq polymerase. During the extension phase of the PCR amplification cycle, cleavage of the probe by Taq polymerase releases the reporter dye from the remainder of the probe (and hence from the quencher moiety) and a sequence-specific fluorescent signal is generated. With each cycle, additional reporter dye molecules are cleaved from their respective probes, and the fluorescence intensity is monitored at regular (six-second) intervals by laser optics built into the ABI PRISM™ 7700 Sequence Detection System. In each assay, a series of parallel reactions containing serial dilutions of mRNA from untreated control samples generates a standard curve that is used to quantitate the percent inhibition after antisense oligonucleotide treatment of test samples.

PCR reagents were obtained from PE-Applied Biosystems, Foster City, CA. RT-PCR reactions were carried out by adding 25 μ L PCR cocktail (1x TAQMAN™ buffer A, 5.5 mM $MgCl_2$, 300 μ M each of dATP, dCTP and dGTP, 600 μ M of dUTP, 100 nM each of forward primer, reverse primer, and probe, 20 Units RNase inhibitor, 1.25 Units AMPLITAQ GOLD™, and 12.5 Units MuLV reverse transcriptase) to 96 well plates

-72-

containing 25 μ L poly(A) mRNA solution. The RT reaction was carried out by incubation for 30 minutes at 48°C. Following a 10 minute incubation at 95°C to activate the AMPLITAQ GOLD™, 40 cycles of a two-step PCR protocol were carried out: 95°C for 15 seconds (denaturation) followed by 60°C for 1.5 minutes (annealing/extension). SRA probes and primers were designed to hybridize to a consensus region of the four human SRA sequences against which antisense oligonucleotides were designed, incorporated herein as SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 and SEQ ID NO:4).

For SRA the PCR primers were:

forward primer: GTGGCCACACAAGGAAGCA (SEQ ID NO: 5)

reverse primer: CTTTCCTCCAGCCCACTGTTC (SEQ ID NO: 6) and

the PCR probe was: FAM-ATGACATCAGCCGACGCCTGGA-TAMRA

(SEQ ID NO: 7) where FAM (PE-Applied Biosystems, Foster City, CA) is the fluorescent reporter dye) and TAMRA (PE-Applied Biosystems, Foster City, CA) is the quencher dye.

For GAPDH the PCR primers were:

forward primer: GAAGGTGAAGGTCGGAGTC (SEQ ID NO: 8)

reverse primer: GAAGATGGTGATGGGATTTTC (SEQ ID NO: 9) and the PCR probe was: 5' JOE-CAAGCTTCCCGTTCTCAGCC- TAMRA 3' (SEQ ID NO: 10) where JOE (PE-Applied Biosystems, Foster City, CA) is the fluorescent reporter dye) and TAMRA (PE-Applied Biosystems, Foster City, CA) is the quencher dye.

Example 14

Northern blot analysis of SRA mRNA levels

Eighteen hours after antisense treatment, cell monolayers were washed twice with cold PBS and lysed in 1 mL RNAZOL™ (TEL-TEST "B" Inc., Friendswood, TX). Total RNA was prepared following manufacturer's recommended protocols. Twenty micrograms of total RNA was fractionated by electrophoresis through 1.2% agarose gels containing 1.1% formaldehyde using a MOPS buffer system (AMRESCO, Inc. Solon, OH). RNA was transferred from the gel to HYBOND™-N+ nylon membranes (Amersham Pharmacia Biotech, Piscataway,

-73-

NJ) by overnight capillary transfer using a Northern/Southern Transfer buffer system (TEL-TEST "B" Inc., Friendswood, TX). RNA transfer was confirmed by UV visualization. Membranes were fixed by UV cross-linking using a STRATALINKER™ UV Crosslinker 2400 (Stratagene, Inc, La Jolla, CA).

Membranes were probed using QUICKHYB™ hybridization solution (Stratagene, La Jolla, CA) using manufacturer's recommendations for stringent conditions with a SRA specific probe prepared by PCR using the forward primer GTGGCCACACAAGGAAGCA (SEQ ID NO: 5) and the reverse primer CTTTCCTCCAGCCCACTGTTC (SEQ ID NO: 6). To normalize for variations in loading and transfer efficiency membranes were stripped and probed for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) RNA (Clontech, Palo Alto, CA). Hybridized membranes were visualized and quantitated using a PHOSPHORIMAGER™ and IMAGEQUANT™ Software V3.3 (Molecular Dynamics, Sunnyvale, CA). Data was normalized to GAPDH levels in untreated controls.

20 Example 15

Antisense inhibition of SRA mRNA - phosphorothioate oligodeoxynucleotides

In accordance with the present invention, a series of oligonucleotides were designed to target different regions of four sequences encoding human SRA RNA, incorporated herein as SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4). The oligonucleotides are shown in Table 1. Target sites are indicated by nucleotide numbers on the target sequence to which the oligonucleotide binds. All compounds in Table 1 are oligodeoxynucleotides with phosphorothioate backbones (internucleoside linkages) throughout. The compounds were analyzed for effect on SRA mRNA levels by quantitative real-time PCR as described in other examples herein. Data are averages from two experiments. If present, "N.D." indicates "no data".

-74-

Table 1

Inhibition of SRA mRNA levels by phosphorothioate
oligodeoxynucleotides

	ISIS#	TARGET (SEQ ID)	TARGET SITE	SEQUENCE	% Inhibition	SEQ ID NO.
5	30138	1	1	cgagcgcctgggtccgcc	52	11
	30139	1	18	accctcttggttaagcagc	0	12
	30140	1	77	gaggcccaggagaagtct	38	13
	30141	1	114	ggagccttacttgaagga	46	14
10	30142	1	139	accactccccacaggtgg	0	15
	30143	1	148	agaggcaggaccactccc	8	16
	30144	1	158	gctccacgccagaggcag	51	17
	30145	1	168	aaacttggtgggctccacg	82	18
	30146	1	178	ctcgactgggaaacttgt	37	19
15	30147	1	188	gagcctcagactcgactg	35	20
	30148	1	199	ctccatcagtcgagcctc	0	21
	30149	1	208	cagcacatcctccatcag	39	22
	30150	1	220	ttccaaaggtctcagcac	51	23
	30151	1	229	caatgcctgttccaaagg	9	24
20	30152	1	240	cggcagtcttccaatgcc	0	25
	30153	1	250	tgtgtggccacggcagtc	53	26
	30154	1	261	acctgcttccttgtgtgg	68	27
	30155	1	272	tgtcatcacatacctgct	24	28
	30156	1	282	cgtcggctgatgtcatca	39	29
25	30157	1	292	cagtgccaggcgtcggct	34	30
	30158	1	303	tgttcctgcagcagtgcc	61	31
	30159	1	314	ctccagcccactgttcct	0	32
	30160	1	325	tgacaactttcctccagc	85	33
	30161	1	336	tttacaggatttgacaac	0	34
30	30162	1	347	ccattctcttcttttacag	40	35
	30163	1	359	gcaccagtagagccattc	70	36
	30164	1	370	tgaaagctcttgcaccag	36	37
	30165	1	382	ccaccggtggccttgaaag	15	38
	30166	1	394	atctgctgcgtcccaccg	10	39
35	30167	1	403	gtggatgtcatctgctgc	0	40
	30168	1	414	atgagggagcggatg	0	41
	30169	1	425	catggtcaaccatgaggg	0	42
	30170	1	438	ctgacctcagtcacatgg	0	43
	30171	1	450	accatccactgactgacc	0	44
40	30172	1	462	cttttaactcctaccatc	57	45
	30173	1	478	cttttctgcaattaatct	39	46
	30174	1	491	aaaacagactcctctttt	46	47
	30175	1	507	ttggctgcctcctctgaa	42	48

204720-9492550

- 75 -

	30176	1	542	ggttccttctcagctgtgg	51	49
	30177	1	585	ggaaccgaggattatgaa	32	50
	30218	2	1	ccgcgttccttgttgccg	6	51
	30219	2	7	ttccagccgcgttccttg	0	52
5	30220	2	22	aactgcgggcggtcgttc	0	53
	30221	2	47	ggcctgggtctgcagccc	6	54
	30222	2	59	cctgggtccgcccggcctg	14	55
	30223	2	75	tggtaagcagcgagcgcc	26	56
	30224	2	139	ggcccaggagaagtctct	0	57
10	30225	2	167	tgaaggaggtggaggccc	0	58
	30226	2	204	gaccactccccacaggtg	0	59
	30227	2	238	actgggaaacttgtgggc	0	60
	30229	2	273	tcagcacatcctccatca	3	61
	30230	2	310	tgccacggcagtcctcc	48	62
15	30231	2	324	cctgcttccttgtgtggc	34	63
	30232	2	345	gtcggctgatgtcatcac	0	64
	30233	2	383	ctttcctccagcccactg	64	65
	30236	2	456	ctgctgcgtcccaccggt	79	66
	30237	2	471	agcgggtggatgtcatctg	35	67
20	30238	2	492	tcacatggtcaaccatga	7	68
	30239	2	525	ttttaactcctaccatcc	0	69
	30240	2	540	tttctgcaattaatcttt	0	70
	30241	2	561	cctctgaaaacagactcc	29	71
	30242	2	581	tttctcttcattggctgc	62	72
25	30243	2	599	ctcagctgtggctgcaga	0	73
	30244	2	615	gtatggatatggttcttct	6	74
	30245	2	629	ctgctggaagcctggtat	23	75
	30246	2	637	tatgaagcctgctggaag	36	76
	30247	2	667	ggtgtccggtgagtctgg	50	77
30	30248	2	686	tctccaaggcataggaga	20	78
	30249	2	696	tgacagaaggctctccaag	19	79
	30250	2	705	ggagccaagtgcagaag	14	80
	30251	2	720	ggtggtggtaagaaggga	0	81
	30252	2	745	gggtcaggcccagtgagg	0	82
35	30253	2	759	cttcctcataggtgggt	0	83
	30254	2	779	ctctggcccagggtgggac	45	84
	30255	2	792	acacatgaactccctctg	23	85
	30256	2	808	atgcatgttatgagtaac	0	86
	30257	2	835	tttcaccgcagagatgtt	0	87
40	30371	3	56	gagggtccataactaagcg	47	88
	30372	3	103	gaggcccggaaactccact	11	89
	30373	3	140	ttccttgttgctgcgga	28	90
	30374	3	174	cgtatgagaactgcggcg	0	91

20040326

-76-

	30375	3	284	aggcccaggagaagtctc	39	92
	30376	3	361	acgccagaggcaggacca	45	93
	30377	3	400	agtcgagcctcagactcg	28	94
	30378	3	473	acatacctgcttccttgt	0	95
5	30379	3	492	ggcgtcggctgatgtcat	58	96
	30380	3	509	ttcctgcagcagtgccag	55	97
	30381	3	529	aactttcctccagcccac	79	98
	30382	3	547	ttctttacaggatttgac	0	99
	30383	3	581	gcttgaaagctcttgac	0	100
10	30384	3	601	tctgctgcgtcccaccgg	68	101
	30385	3	620	gagggagcgggtggatgtc	52	102
	30386	3	640	tcagtcacatgggtcaacc	50	103
	30387	3	663	ctcctaccatccactgac	54	104
	30388	3	683	ttctgcaattaatctttt	41	105
15	30389	3	702	ctgaaaacagactcctct	44	106
	30390	3	720	cttcattggctgcctcct	80	107
	30391	3	756	tggtatggttcttctcag	42	108
	30392	3	772	tgctggaagcctggatg	72	109
	30393	3	791	aaccgaggattatgaagc	43	110
20	30394	3	827	ggcaccggaaggagatgg	57	111
	30395	3	847	gacaccagagggactagc	27	112
	30396	3	867	cagggagcagggcagtcg	65	113
	30397	3	885	cagaggtttgcagataca	65	114
	30398	3	903	tgaatggagaagggagaa	25	115
25	30399	3	922	ccagcagatcccttcctg	0	116
	30400	3	944	gtaggcagtagtctgact	58	117
	30401	3	977	ggatgcttggttcagtcta	0	118
	30402	3	992	gctccgcccagcacagga	63	119
	30403	3	1007	catccaaacacagctgct	61	120
30	30404	3	1027	ttctcatgctgaaatgaa	26	121
	30309	4	269	cccggcttcacgtacagc	37	122
	30310	4	288	ccagccgcgttccttggt	31	123
	30311	4	323	gtctgcagcccgtatgag	0	124
	30312	4	339	gggtccgcggcctgggt	0	125
35	30313	4	359	ttggtaagcagcgagcgc	44	126
	30314	4	448	aaggaggtggaggcccca	28	127
	30315	4	462	gggagccttacttgaagg	12	128
	30316	4	498	gccagaggcaggaccact	28	129
	30317	4	549	atcctccatcagtcgagc	0	130
40	30318	4	566	tccaaaggtctcagcaca	74	131
	30319	4	600	ccttggtgtggccacggca	57	132
	30320	4	616	catcacatacctgcttcc	32	133
	30321	4	635	gccaggcgtcggctgatg	62	134

-77-

	30322	4	669	caacttttcctccagccca	59	135
	30323	4	703	ccagtagagccattctct	17	136
	30324	4	738	tgctgcgtcccaccggtg	73	137
	30328	4	739	gctgtggctgcagatttc	52	138
5	30325	4	773	acatggtcaaccatgagg	46	139
	30326	4	807	tttaactcctaccatcca	11	140
	30327	4	842	tctgaaaacagactcctc	1	141
	30329	4	913	cctgctggaagcctggta	0	142
	30330	4	962	atttgaggagatggtgtc	6	143
10	30331	4	997	ctttcctttttgttgaggg	0	144
	30332	4	1032	gtatctcaagggtgatac	40	145
	30333	4	1067	ccccactcaatgtggttc	69	146

As shown in Table 1, SEQ ID NOS 11, 13, 14, 17, 18, 15 19, 20, 22, 23, 26, 27, 29, 30, 31, 33, 35, 36, 37, 45, 46, 47, 48, 49, 50, 62, 63, 65, 66, 67, 72, 76, 77, 84, 88, 92, 93, 96, 97, 98, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 113, 114, 117, 119, 120, 122, 123, 126, 131, 132, 133, 134, 135, 137, 138, 139, 145 and 146 demonstrated 20 at least 30% inhibition of SRA mRNA levels in this assay and are therefore preferred.

Example 16:**Antisense inhibition of SRA - phosphorothioate 2'-MOE gapmer oligonucleotides**

25 In accordance with the present invention, a second series of oligonucleotides targeted to human SRA were synthesized. The oligonucleotide sequences are shown in Table 2. Target sites are indicated by nucleotide numbers, as given in the target sequence, to which the 30 oligonucleotide binds.

All compounds in Table 2 are chimeric oligonucleotides ("gapmers") 18 nucleotides in length, composed of a central "gap" region consisting of ten 2'-deoxynucleotides, which is flanked on both sides (5' and 3' directions) by four- 35 nucleotide "wings". The wings are composed of 2'-methoxyethyl (2'-MOE)nucleotides. The internucleoside (backbone) linkages are phosphorothioate (P=S) throughout

-78-

the oligonucleotide. Cytidine residues in the 2'-MOE wings are 5-methylcytidines.

Data were obtained by real-time quantitative PCR as described in other examples herein and are averaged from 5 two experiments. If present, "N.D." indicates "no data".

Table 2

Inhibition of SRA mRNA levels by chimeric phosphorothioate oligonucleotides having 2'-MOE wings and a deoxy gap

10	ISIS#	TARGET (SEQ ID)	TARGET SITE	SEQUENCE	% Inhibition	SEQ ID NO.
	30178	1	1	cgagcgcctgggtccgcc	54	11
	30179	1	18	accctcttggttaagcagc	0	12
	30180	1	77	gaggcccaggagaagtct	44	13
15	30181	1	114	ggagccttacttgaagga	38	14
	30182	1	139	accactccccacaggtgg	49	15
	30183	1	148	agaggcaggaccactccc	0	16
	30184	1	158	gctccacgccagaggcag	44	17
	30185	1	168	aaacttgtgggctccacg	48	18
20	30186	1	178	ctcgactgggaaacttgt	39	19
	30187	1	188	gagcctcagactcgactg	24	20
	30188	1	199	ctccatcagtcgagcctc	28	21
	30189	1	208	cagcacatcctccatcag	0	22
	30190	1	220	ttccaaaggtctcagcac	44	23
25	30191	1	229	caatgcctgttccaaagg	29	24
	30192	1	240	cggcagtccttccaatgcc	0	25
	30193	1	250	tgtgtggccacggcagtc	0	26
	30194	1	261	acctgcttccttgtgtgg	0	27
	30195	1	272	tgtcatcacatacctgct	0	28
30	30196	1	282	cgtcggctgatgtcatca	57	29
	30197	1	292	cagtgccaggcgctcggct	39	30
	30198	1	303	tgttcctgcagcagtgcc	0	31
	30199	1	314	ctccagcccactgttcct	27	32
	30200	1	325	tgacaactttcctccagc	18	33
35	30201	1	336	tttacaggtattgacaac	0	34
	30202	1	347	ccattctcttctttacag	29	35
	30203	1	359	gcaccagtagagccattc	22	36
	30204	1	370	tgaaagctcttgaccag	27	37
	30205	1	382	ccaccggtggcttgaaag	39	38
40	30206	1	394	atctgctgcgtcccaccg	22	39
	30207	1	403	gtggatgtcatctgctgc	63	40

-79-

	30208	1	414	atgagggagcggtggatg	43	41
	30209	1	425	catggtcaaccatgaggg	56	42
	30210	1	438	ctgacctcagtcacatgg	41	43
	30211	1	450	accatccactgactgacc	0	44
5	30212	1	462	cttttaactcctaccatc	42	45
	30213	1	478	cttttctgcaattaatct	0	46
	30214	1	491	aaaacagactcctctttt	31	47
	30215	1	507	ttggctgcctcctctgaa	68	48
	30216	1	542	ggttcttctcagctgtgg	0	49
10	30217	1	585	ggaaccgaggattatgaa	71	50
	30258	2	1	ccgcgttccttgttgccg	53	51
	30259	2	7	ttccagccgcgttccttg	0	52
	30260	2	22	aactgcggcggtcggttc	0	53
	30261	2	47	ggcctgggtctgcagccc	37	54
15	30262	2	59	cctgggtccgccggcctg	27	55
	30263	2	75	tggtaagcagcgagcgcc	0	56
	30264	2	139	ggcccaggagaagtctct	43	57
	30265	2	167	tgaaggaggtggaggccc	29	58
	30266	2	204	gaccactccccacaggtg	28	59
20	30267	2	238	actgggaaacttgtgggc	7	60
	30269	2	273	tcagcacatcctccatca	0	61
	30270	2	310	tggccacggcagtccttc	50	62
	30271	2	324	cctgcttccttgtgtggc	59	63
	30272	2	345	gtcggctgatgtcatcac	38	64
25	30273	2	383	ctttcctccagcccactg	0	65
	30276	2	456	ctgctgctgccaccggt	83	66
	30277	2	471	agcgggtggatgtcatctg	67	67
	30278	2	492	tcacatggtcaaccatga	62	68
	30279	2	525	ttttaactcctaccatcc	0	69
30	30280	2	540	tttctgcaattaatcttt	18	70
	30281	2	561	cctctgaaaacagactcc	3	71
	30282	2	581	tttctcttcattggctgc	77	72
	30283	2	599	ctcagctgtggctgcaga	55	73
	30284	2	615	gtatggtatggttcttct	56	74
35	30285	2	629	ctgctggaagcctggtat	51	75
	30286	2	637	tatgaagcctgctggaag	22	76
	30287	2	667	ggtgtccggtgagtcctgg	0	77
	30288	2	686	tctccaaggcataggaga	0	78
	30289	2	696	tgacagaaggtctccaag	73	79
40	30290	2	705	ggagccaagtgcagaag	27	80
	30291	2	720	ggtggtggttaagaaggga	34	81
	30292	2	745	gggtcaggcccagtgggga	0	82
	30293	2	759	cttccctcataggtgggt	51	83

204720 9492650

- 80 -

	30294	2	779	ctctggcccaggtgggac	50	84
	30295	2	792	acacatgaactccctctg	41	85
	30296	2	808	atgcatgttatgagtaac	70	86
	30297	2	835	tttcaccgcagagatgtt	35	87
5	30407	3	56	gagggtccataactaagcg	70	88
	30408	3	103	gaggcccgaactccact	53	89
	30409	3	140	ttccttggtgcctgcgga	69	90
	30410	3	174	cgtatgagaactgcggcg	88	91
	30411	3	284	aggcccaggagaagtctc	79	92
10	30412	3	361	acgccagaggcaggacca	57	93
	30413	3	400	agtcgagcctcagactcg	71	94
	30414	3	473	acatacctgcttccttgt	22	95
	30415	3	492	ggcgtcggctgatgtcat	73	96
	30416	3	509	ttcctgcagcagtgccag	85	97
15	30417	3	529	aactttcctccagcccac	69	98
	30418	3	547	ttctttacaggatttgac	70	99
	30419	3	581	gcttgaaagctcttgac	77	100
	30420	3	601	tctgctgcgtcccaccgg	82	101
	30421	3	620	gagggagcggatgtc	35	102
20	30422	3	640	tcagtcacatgggtcaacc	78	103
	30423	3	663	ctcctaccatccactgac	56	104
	30424	3	683	ttctgcaattaatctttt	67	105
	30425	3	702	ctgaaaacagactcctct	76	106
	30426	3	720	cttcattggctgcctcct	81	107
25	30427	3	756	tggtatggttcttctcag	90	108
	30428	3	772	tgctggaagcctggatg	88	109
	30429	3	791	aaccgaggattatgaagc	78	110
	30430	3	827	ggcaccggaaggagatgg	48	111
	30431	3	847	gacaccagagggactagc	25	112
30	30432	3	867	cagggagcagggcagtcg	43	113
	30433	3	885	cagaggtttgagataca	83	114
	30434	3	903	tgaatggagaaggagaa	56	115
	30435	3	922	ccagcagatcccttcctg	32	116
	30436	3	944	gtaggcagtagtctgact	75	117
35	30437	3	977	ggatgcttggtcagtcta	21	118
	30438	3	992	gctccgcccagcacagga	79	119
	30439	3	1007	catccaaacacagctgct	72	120
	30440	3	1027	ttctcatgctgaaatgaa	62	121
	30345	4	269	cccggcttcacgtacagc	57	122
40	30346	4	288	ccagccgcgttccttggt	73	123
	30347	4	323	gtctgcagcccgtatgag	36	124
	30348	4	339	gggtccgcccggcctgggt	23	125
	30349	4	359	ttggtaagcagcgagcgc	83	126

Patent 2000/0058332

-81-

	30350	4	448	aaggaggtggaggcccca	52	127
	30351	4	462	gggagccttacttgaagg	3	128
	30352	4	498	gccagaggcaggaccact	82	129
	30353	4	549	atcctccatcagtcgagc	0	130
5	30354	4	566	tccaaaggtctcagcaca	80	131
	30355	4	600	ccttgtgtggccacggca	82	132
	30356	4	616	catcacatacctgcttcc	35	133
	30357	4	635	gccaggcgctcggtgatg	64	134
	30358	4	669	caactttcctccagccca	41	135
10	30359	4	703	ccagtagagccattctct	70	136
	30360	4	738	tgctgcgtcccaccggtg	87	137
	30364	4	739	gctgtggctgcagatttc	76	138
	30361	4	773	acatggtcaaccatgagg	87	139
	30362	4	807	tttaactcctaccatcca	14	140
15	30363	4	842	tctgaaaacagactcctc	38	141
	30365	4	913	cctgctggaagcctggta	74	142
	30366	4	962	atttgaggagatggtgtc	56	143
	30367	4	997	ctttccttttggtgaggg	60	144
	30368	4	1032	gtatctcaagggtgatac	72	145
20	30369	4	1067	ccccactcaatgtggttc	81	146

As shown in Table 2, SEQ ID NOs 11, 13, 14, 15, 17, 18, 19, 23, 29, 30, 38, 40, 41, 42, 43, 45, 47, 48, 50, 51, 54, 57, 62, 63, 64, 66, 67, 68, 72, 73, 74, 75, 79, 81, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 113, 114, 115, 116, 117, 119, 120, 121, 122, 123, 124, 126, 127, 129, 131, 132, 133, 134, 135, 136, 137, 138, 139, 141, 142, 143, 144, 145 and 146 demonstrated at least 30% inhibition of SRA expression in this experiment and are therefore preferred.

What is claimed is:

1. An antisense compound 8 to 30 nucleobases in length targeted to human SRA or a nucleic acid molecule encoding human SRA, wherein said antisense compound specifically hybridizes with and decreases the function or amount of human
5 SRA.
2. The antisense compound of claim 1 which is an antisense oligonucleotide.
3. The antisense compound of claim 2 wherein the antisense oligonucleotide has a sequence comprising SEQ ID NO:
10 11, 13, 14, 15, 17, 18, 19, 20, 22, 23, 26, 27, 29, 30, 31, 33, 35, 36, 37, 38, 40, 41, 42, 43, 45, 46, 47, 48, 49, 50, 62, 63, 64, 65, 66, 67, 68, 72, 73, 74, 75, 76, 77, 79, 81, 83, 84, 85, 86, 87, 51, 54, 57, 88, 89, 90, 91, 92, 93, 94, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108,
15 109, 110, 111, 113, 114, 115, 116, 117, 119, 120, 121, 122, 123, 124, 126, 127, 129, 131, 132, 133, 134, 135, 136, 137, 139, 141, 138, 142, 143, 144, 145 or 146.
4. The antisense compound of claim 2 wherein the antisense oligonucleotide has a sequence comprising SEQ ID
20 NO: 11, 13, 14, 17, 18, 19, 23, 29, 30, 45, 47, 48, 50, 62, 63, 66, 67, 72, 84, 88, 92, 93, 96, 97, 98, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 113, 114, 117, 119, 120, 122, 123, 126, 131, 132, 133, 134, 135, 137, 139, 138, 145 or 146.
- 25 5. The antisense compound of claim 2 wherein the antisense oligonucleotide comprises at least one modified internucleoside linkage.
6. The antisense compound of claim 5 wherein the modified internucleoside linkage is a phosphorothioate
30 linkage.
7. The antisense compound of claim 2 wherein the antisense oligonucleotide comprises at least one modified sugar moiety.

8. The antisense compound of claim 7 wherein the modified sugar moiety is a 2'-O-methoxyethyl sugar moiety.

9. The antisense compound of claim 2 wherein the antisense oligonucleotide comprises at least one modified nucleobase.

10. The antisense compound of claim 9 wherein the modified nucleobase is a 5-methylcytosine.

11. The antisense compound of claim 1 wherein the antisense oligonucleotide is a chimeric oligonucleotide.

10 12. A composition comprising the antisense compound of
claim 1 and a pharmaceutically acceptable carrier or diluent.

13. The composition of claim 12 further comprising a colloidal dispersion system.

14. The composition of claim 12 wherein the antisense
15 compound is an antisense oligonucleotide.

15. A method of decreasing the function or amount of SRA in human cells or tissues comprising contacting said cells or tissues with the antisense compound of claim 1 so that the function or amount of SRA is inhibited.

20 16. A method of treating a human having a disease or condition associated with SRA comprising administering to said animal a therapeutically or prophylactically effective amount of the antisense compound of claim 1 so that the function or amount of SRA is inhibited.

25 17. The method of claim 16 wherein the disease or
condition is associated with steroid hormones.

SEQUENCE LISTING

<110> Lex M. Cowser
C. Frank Bennett
Bert W. O'Malley
ISIS PHARMACEUTICALS, INC.
BAYLOR COLLEGE OF MEDICINE

<120> ANTISENSE MODULATION OF SRA EXPRESSION

<130> RTSP-0058

<150> US 09/280,409

<151> 1999-03-29

<160> 146

<210> 1

<211> 626

<212> DNA

<213> Homo sapiens

<400> 1

```

ggcggaccca ggcgctcgct gcttaccaag agggtagccg caccacagga tggatcccc 60
agagtccccg catcagagac ttctcctggg cctcccccaa tggggcctcc acctccttca 120
agtaaggctc ccaggtcccc acctgtgggg agtggtcctg cctctggcgt ggagcccaca 180
agtttcccag tcgagtctga ggctcgactg atggaggatg tgctgagacc ttggaacag 240
gcattggaag actgccgtgg ccacacaagg aagcaggatg gtgatgacat cagccgacgc 300
ctggcactgc tgcaggaaca gtgggctgga ggaaagtgt caatacctgt aaagaagaga 360
atggctctac tgggtcaaga gctttcaagc caccggtggg acgcagcaga tgacatccac 420
cgctccctca tgggtgacca tgtgactgag gtcagtcagt ggatggtagg agttaaaga 480
ttaattgcag aaaagaggag tctgttttca gaggaggcag ccaatgaaga gaaatctgca 540
gccacagctg agaagaacca taccatacca ggcttccagc aggcttcata atcctcggtt 600
ccccagactc accggacacc atctcc 626

```

<210> 2

<211> 859

<212> DNA

<213> Homo sapiens

<400> 2

```

cggcaacaag gaacgcggct ggaacgaccc gccgcagttc tcatacgggc tgcagaccca 60
ggccggcgga ccagggcgct cgctgcttac caagagggtc gccgcacccc aggatggatc 120
ccccagagtc ccgcacag agacttctcc tgggcctccc ccaatggggc ctccacctcc 180
ttcaagtaag gctcccaggc cccacactgt ggggagtggc cctgcctctg gcgtggagcc 240
cacaagtttc ccagtcgagt ctgaggctcg actgatggag gatgtgctga gacctttgga 300
acaggcattg gaagactgcc gtggccacac aaggaagcag gtatgtgatg acatcagccg 360

```


acgcctggca ctgctgcagg aacagtgggc tggaggaaag ttgtcaatac ctgtaaagaa 420
gagaatggct ctactggtgc aagagctttc aagccaccgg tgggacgcag cagatgacat 480
ccaccgctcc ctcatggttg accatgtgac tgaggtcagt cagtggatgg taggagttaa 540
aagattaatt gcagaaaaga ggagtctgtt ttcagaggag gcagccaatg aagagaaatc 600
tgcagccaca gctgagaaga accataccat accaggcttc cagcaggctt cataatcctc 660
ggttccccag actcaccgga caccatctcc tatgccttgg agaccttctg tcacttggct 720
cccttcttac caccaccaag actgtccac tgggcctgac ccacctatga ggaagaagt 780
cccacctggg ccagagggag ttcattgtgt actcataaca tgcatttcaa taaaaacatc 840
tctgcggtga aaaaaaaaaa 859

<210> 3
<211> 1086
<212> DNA
<213> Homo sapiens

<400> 3
gtagcgaccg gcgctcagct ggcgtcgccc cggcctaggc tgggggcggt tgcggcgctt 60
agtatggacc ctctgtctcc cccagcccca gtatcagcta acagtggagt tccgggcctc 120
gcttcacaca tccctcgctt ccgcaggcaa caaggaacgc ggctggaacg acccgccgca 180
gttctcatac gggctgcaga cccaggccgg cggaccagcgc cgctcgctgc ttaccaagag 240
ggtagccgca cccaggatg gatccccag agtccccgca tcagagactt ctccctgggccc 300
tcccccaatg gggcctccac ctccctcaag taaggctccc aggtccccac ctgtggggag 360
tggctcctgcc tctggcgtgg agcccacaag tttcccagtc gagtctgagg ctcgactgat 420
ggaggatgtg ctgagacctt tggaacaggc attggaagac tgccgtggcc acacaaggaa 480
gcaggatatgt gatgacatca gccgacgcct ggcactgctg caggaaacagt gggctggagg 540
aaagttgtca atacctgtaa agaagagaat ggctctactg gtgcaagagc tttcaagcca 600
ccggtgggac gcagcagatg acatccaccg ctccctcatg gttgaccatg tgactgaggt 660
cagtcagtgg atggtaggag ttaaaagatt aattgcagaa aagaggagtc tgttttcaga 720
ggaggcagcc aatgaagaga aatctgcagc cacagctgag aagaaccata ccataccagg 780
cttcacagcag gcttcataat cctcggttcc ccagactcac cggacaccat ctccctccgg 840
tgccaagcta gtccctctgg tgtcctcgac tgccctgctc cctgtgtatc tgcaaacctc 900
tgttctccct tctccattca tcaggaaggg atctgtctgg taaagtcaga ctactgccta 960
ccactttttc ccaaagtaga ctgaacaagc atcctgtgct gggcggagca gctgtgtttg 1020
gatggtttca tttcagcatg agaacagact caaatagaac gggccggaat tccgccgata 1080
ctgacg 1086

<210> 4
 <211> 1123
 <212> DNA
 <213> Homo sapiens

<400> 4
 actcctggag cccgtcagta tcggcggccc tcatcacgtc cccacactgg ctgtacagga 60
 tcttgccatc cccactcgg tcaaacagct cgaaggcctc cttgaactcc tccagctggt 120
 ccttggttaa ctcgatcacc actttggaga gatcgactgg aggctcctgg gttttctgag 180
 gggcctgggg gacagctggc tcagctttgg tcttggtgg aggagcccct gctgctgctg 240
 gcttagcagc aggtttggag atggaggagc tgtacgtgaa gccgggcaac aaggaacgcg 300
 gctggaacga cccgccgag ttctcatagc ggctgcagac ccaggccggc ggaccagggc 360
 gctcgtgct taccaagagg gtagccgcac cccaggatgg atccccaga gtccccgcat 420
 cagagacttc tcttgggcct cccccaatgg ggctccacc tccttcaagt aaggctccca 480
 ggtccccacc tgtggggagt ggtcctgcct ctggcgtgga gccacaagt ttcccagtcg 540
 agtctgaggc tcgactgatg gaggatgtgc tgagacctt ggaacaggca ttggaagact 600
 gccgtggcca cacaaggaag caggtatgtg atgacatcag ccgacgcctg gcaactgctgc 660
 aggaacagtg ggctggagga aagttgtcaa tacctgtaaa gaagagaatg gctctactgg 720
 tgcaagagct ttcaagccac cgggtgggacg cagcagatga catccaccgc tccctcatgg 780
 ttgacctgt gactgaggtc agtcagtgga tggtaggagt taaaagatta attgcagaaa 840
 agaggagtct gttttcagag gaggcagcca atgaagagaa atctgcagcc acagctgaga 900
 agaaccatac cataccaggc ttccagcagg cttcataatc ctcggttccc cagactcacc 960
 ggacaccatc tcgtcaaata gaacggggag acttttccct caacaaaagg aaagacagtc 1020
 ctatttgcac tgtatcacc ttgagatact actgttacag agattagaac cacattgagt 1080
 ggggttttct gtgtaaatcg aaggagaaaa agaccagatt act 1123

<210> 5
 <211> 19
 <212> DNA
 <213> Artificial Sequence

<223> PCR Primer

<400> 5
 gtggccacac aaggaagca 19

<210> 6
 <211> 21
 <212> DNA
 <213> Artificial Sequence

<223> PCR Primer
 <400> 6
 ctttcctcca gccactgtt c 21
 <210> 7
 <211> 22
 <212> DNA
 <213> Artificial Sequence
 <223> PCR Probe
 <400> 7
 atgacatcag ccgacgcctg ga 22
 <210> 8
 <211> 19
 <212> DNA
 <213> Artificial Sequence
 <223> PCR Primer
 <400> 8
 gaaggtgaag gtcggagtc 19
 <210> 9
 <211> 20
 <212> DNA
 <213> Artificial Sequence
 <223> PCR Primer
 <400> 9
 gaagatggtg atgggatttc 20
 <210> 10
 <211> 20
 <212> DNA
 <213> Artificial Sequence
 <223> PCR Probe
 <400> 10
 caagcttccc gttctcagcc 20
 <210> 11
 <211> 18
 <212> DNA
 <213> Artificial Sequence
 <223> Antisense Oligonucleotide
 <400> 11
 cgagcgcctg ggtccgcc 18
 <210> 12
 <211> 18

<212> DNA
<213> Artificial Sequence

<223> Antisense Oligonucleotide

<400> 12
accctcttgg taagcagc 18

<210> 13
<211> 18
<212> DNA
<213> Artificial Sequence

<223> Antisense Oligonucleotide

<400> 13
gaggcccagg agaagtct 18

<210> 14
<211> 18
<212> DNA
<213> Artificial Sequence

<223> Antisense Oligonucleotide

<400> 14
ggagccttac ttgaagga 18

<210> 15
<211> 18
<212> DNA
<213> Artificial Sequence

<223> Antisense Oligonucleotide

<400> 15
accactcccc acaggtgg 18

<210> 16
<211> 18
<212> DNA
<213> Artificial Sequence

<223> Antisense Oligonucleotide

<400> 16
agaggcagga ccactccc 18

<210> 17
<211> 18
<212> DNA
<213> Artificial Sequence

<223> Antisense Oligonucleotide

<400> 17
gctccacgcc agaggcag 18

<210> 18
 <211> 18
 <212> DNA
 <213> Artificial Sequence

 <223> Antisense Oligonucleotide

 <400> 18
 aaacttgtgg gctccacg 18

<210> 19
 <211> 18
 <212> DNA
 <213> Artificial Sequence

 <223> Antisense Oligonucleotide

 <400> 19
 ctcgactggg aaacttgt 18

<210> 20
 <211> 18
 <212> DNA
 <213> Artificial Sequence

 <223> Antisense Oligonucleotide

 <400> 20
 gagcctcaga ctcgactg 18

<210> 21
 <211> 18
 <212> DNA
 <213> Artificial Sequence

 <223> Antisense Oligonucleotide

 <400> 21
 ctccatcagt cgagcctc 18

<210> 22
 <211> 18
 <212> DNA
 <213> Artificial Sequence

 <223> Antisense Oligonucleotide

 <400> 22
 cagcacatcc tccatcag 18

<210> 23
 <211> 18
 <212> DNA
 <213> Artificial Sequence

 <223> Antisense Oligonucleotide

 <400> 23

ttccaaaggt ctcagcac

18

<210> 24

<211> 18

<212> DNA

<213> Artificial Sequence

<223> Antisense Oligonucleotide

<400> 24

caatgcctgt tccaaagg

18

<210> 25

<211> 18

<212> DNA

<213> Artificial Sequence

<223> Antisense Oligonucleotide

<400> 25

cggcagtctt ccaatgcc

18

<210> 26

<211> 18

<212> DNA

<213> Artificial Sequence

<223> Antisense Oligonucleotide

<400> 26

tgtgtggcca cggcagtc

18

<210> 27

<211> 18

<212> DNA

<213> Artificial Sequence

<223> Antisense Oligonucleotide

<400> 27

acctgcttcc ttgtgtgg

18

<210> 28

<211> 18

<212> DNA

<213> Artificial Sequence

<223> Antisense Oligonucleotide

<400> 28

tgtcatcaca tacctgct

18

<210> 29

<211> 18

<212> DNA

<213> Artificial Sequence

<223> Antisense Oligonucleotide
 <400> 29
 cgtcggctga tgtcatca 18

 <210> 30
 <211> 18
 <212> DNA
 <213> Artificial Sequence

 <223> Antisense Oligonucleotide
 <400> 30
 cagtgccagg cgtcggct 18

 <210> 31
 <211> 18
 <212> DNA
 <213> Artificial Sequence

 <223> Antisense Oligonucleotide
 <400> 31
 tgttcctgca gcagtgcc 18

 <210> 32
 <211> 18
 <212> DNA
 <213> Artificial Sequence

 <223> Antisense Oligonucleotide
 <400> 32
 ctccagccca ctgttcct 18

 <210> 33
 <211> 18
 <212> DNA
 <213> Artificial Sequence

 <223> Antisense Oligonucleotide
 <400> 33
 tgacaacttt cctccagc 18

 <210> 34
 <211> 18
 <212> DNA
 <213> Artificial Sequence

 <223> Antisense Oligonucleotide
 <400> 34
 tttacaggta ttgacaac 18

 <210> 35
 <211> 18

<212> DNA
 <213> Artificial Sequence
 <223> Antisense Oligonucleotide
 <400> 35
 ccattctctt ctttacag 18

<210> 36
 <211> 18
 <212> DNA
 <213> Artificial Sequence
 <223> Antisense Oligonucleotide
 <400> 36
 gcaccagtag agccattc 18

<210> 37
 <211> 18
 <212> DNA
 <213> Artificial Sequence
 <223> Antisense Oligonucleotide
 <400> 37
 tgaaagctct tgcaccag 18

<210> 38
 <211> 18
 <212> DNA
 <213> Artificial Sequence
 <223> Antisense Oligonucleotide
 <400> 38
 ccaccggtgg cttgaaag 18

<210> 39
 <211> 18
 <212> DNA
 <213> Artificial Sequence
 <223> Antisense Oligonucleotide
 <400> 39
 atctgctgcg tcccaccg 18

<210> 40
 <211> 18
 <212> DNA
 <213> Artificial Sequence
 <223> Antisense Oligonucleotide
 <400> 40
 gtggatgtca tctgctgc 18

<210> 41
<211> 18
<212> DNA
<213> Artificial Sequence

<223> Antisense Oligonucleotide

<400> 41
atgagggagc ggtggatg 18

<210> 42
<211> 18
<212> DNA
<213> Artificial Sequence

<223> Antisense Oligonucleotide

<400> 42
catggtcaac catgaggg 18

<210> 43
<211> 18
<212> DNA
<213> Artificial Sequence

<223> Antisense Oligonucleotide

<400> 43
ctgacctcag tcacatgg 18

<210> 44
<211> 18
<212> DNA
<213> Artificial Sequence

<223> Antisense Oligonucleotide

<400> 44
accatccact gactgacc 18

<210> 45
<211> 18
<212> DNA
<213> Artificial Sequence

<223> Antisense Oligonucleotide

<400> 45
cttttaactc ctaccatc 18

<210> 46
<211> 18
<212> DNA
<213> Artificial Sequence

<223> Antisense Oligonucleotide

<400> 46

cttttctgca attaatct 18

<210> 47
 <211> 18
 <212> DNA
 <213> Artificial Sequence

<223> Antisense Oligonucleotide

<400> 47
 aaaacagact cctctttt 18

<210> 48
 <211> 18
 <212> DNA
 <213> Artificial Sequence

<223> Antisense Oligonucleotide

<400> 48
 ttggctgcct cctctgaa 18

<210> 49
 <211> 18
 <212> DNA
 <213> Artificial Sequence

<223> Antisense Oligonucleotide

<400> 49
 gggtcttctc agctgtgg 18

<210> 50
 <211> 18
 <212> DNA
 <213> Artificial Sequence

<223> Antisense Oligonucleotide

<400> 50
 ggaaccgagg attatgaa 18

<210> 51
 <211> 18
 <212> DNA
 <213> Artificial Sequence

<223> Antisense Oligonucleotide

<400> 51
 ccgcgttcct tggtgccg 18

<210> 52
 <211> 18
 <212> DNA
 <213> Artificial Sequence

<223> Antisense Oligonucleotide

<400> 52
ttccagccgc gttccttg 18

<210> 53
<211> 18
<212> DNA
<213> Artificial Sequence

<223> Antisense Oligonucleotide

<400> 53
aactgcggcg ggtcgttc 18

<210> 54
<211> 18
<212> DNA
<213> Artificial Sequence

<223> Antisense Oligonucleotide

<400> 54
ggcctgggtc tgcagccc 18

<210> 55
<211> 18
<212> DNA
<213> Artificial Sequence

<223> Antisense Oligonucleotide

<400> 55
cctgggtccg ccggcctg 18

<210> 56
<211> 18
<212> DNA
<213> Artificial Sequence

<223> Antisense Oligonucleotide

<400> 56
tggttaagcag cgagcgcc 18

<210> 57
<211> 18
<212> DNA
<213> Artificial Sequence

<223> Antisense Oligonucleotide

<400> 57
ggcccaggag aagtctct 18

<210> 58
<211> 18

<212> DNA
 <213> Artificial Sequence

 <223> Antisense Oligonucleotide

 <400> 58
 tgaaggaggt ggaggccc 18

 <210> 59
 <211> 18
 <212> DNA
 <213> Artificial Sequence

 <223> Antisense Oligonucleotide

 <400> 59
 gaccactccc cacaggtg 18

 <210> 60
 <211> 18
 <212> DNA
 <213> Artificial Sequence

 <223> Antisense Oligonucleotide

 <400> 60
 actgggaaac ttgtgggc 18

 <210> 61
 <211> 18
 <212> DNA
 <213> Artificial Sequence

 <223> Antisense Oligonucleotide

 <400> 61
 tcagcacatc ctccatca 18

 <210> 62
 <211> 18
 <212> DNA
 <213> Artificial Sequence

 <223> Antisense Oligonucleotide

 <400> 62
 tggccacggc agtcttcc 18

 <210> 63
 <211> 18
 <212> DNA
 <213> Artificial Sequence

 <223> Antisense Oligonucleotide

 <400> 63
 cctgcttcct tgtgtggc 18

<210>	64	
<211>	18	
<212>	DNA	
<213>	Artificial Sequence	
<223>	Antisense Oligonucleotide	
<400>	64	
gtcggctgat	gtcatcac	18
<210>	65	
<211>	18	
<212>	DNA	
<213>	Artificial Sequence	
<223>	Antisense Oligonucleotide	
<400>	65	
ctttcctcca	gcccactg	18
<210>	66	
<211>	18	
<212>	DNA	
<213>	Artificial Sequence	
<223>	Antisense Oligonucleotide	
<400>	66	
ctgctgcgtc	ccaccggt	18
<210>	67	
<211>	18	
<212>	DNA	
<213>	Artificial Sequence	
<223>	Antisense Oligonucleotide	
<400>	67	
agcgggtggat	gtcatctg	18
<210>	68	
<211>	18	
<212>	DNA	
<213>	Artificial Sequence	
<223>	Antisense Oligonucleotide	
<400>	68	
tcacatggtc	aaccatga	18
<210>	69	
<211>	18	
<212>	DNA	
<213>	Artificial Sequence	
<223>	Antisense Oligonucleotide	
<400>	69	

ttttaactcc taccatcc

18

<210> 70

<211> 18

<212> DNA

<213> Artificial Sequence

<223> Antisense Oligonucleotide

<400> 70

tttctgcaat taatcttt

18

<210> 71

<211> 18

<212> DNA

<213> Artificial Sequence

<223> Antisense Oligonucleotide

<400> 71

cctctgaaaa cagactcc

18

<210> 72

<211> 18

<212> DNA

<213> Artificial Sequence

<223> Antisense Oligonucleotide

<400> 72

tttctcttca ttggctgc

18

<210> 73

<211> 18

<212> DNA

<213> Artificial Sequence

<223> Antisense Oligonucleotide

<400> 73

ctcagctgtg gctgcaga

18

<210> 74

<211> 18

<212> DNA

<213> Artificial Sequence

<223> Antisense Oligonucleotide

<400> 74

gtatggtatg gttcttct

18

<210> 75

<211> 18

<212> DNA

<213> Artificial Sequence

<223> Antisense Oligonucleotide
 <400> 75
 ctgctggaag cctggtat 18

 <210> 76
 <211> 18
 <212> DNA
 <213> Artificial Sequence

 <223> Antisense Oligonucleotide
 <400> 76
 tatgaagcct gctggaag 18

 <210> 77
 <211> 18
 <212> DNA
 <213> Artificial Sequence

 <223> Antisense Oligonucleotide
 <400> 77
 ggtgtccggt gagtctgg 18

 <210> 78
 <211> 18
 <212> DNA
 <213> Artificial Sequence

 <223> Antisense Oligonucleotide
 <400> 78
 tctccaaggc ataggaga 18

 <210> 79
 <211> 18
 <212> DNA
 <213> Artificial Sequence

 <223> Antisense Oligonucleotide
 <400> 79
 tgacagaagg tctccaag 18

 <210> 80
 <211> 18
 <212> DNA
 <213> Artificial Sequence

 <223> Antisense Oligonucleotide
 <400> 80
 ggagccaagt gacagaag 18

 <210> 81
 <211> 18

<212> DNA
<213> Artificial Sequence

<223> Antisense Oligonucleotide

<400> 81
ggtggtggta agaagga 18

<210> 82
<211> 18
<212> DNA
<213> Artificial Sequence

<223> Antisense Oligonucleotide

<400> 82
gggtcaggcc cagtggga 18

<210> 83
<211> 18
<212> DNA
<213> Artificial Sequence

<223> Antisense Oligonucleotide

<400> 83
cttccctcat aggtgggt 18

<210> 84
<211> 18
<212> DNA
<213> Artificial Sequence

<223> Antisense Oligonucleotide

<400> 84
ctctggccca ggtgggac 18

<210> 85
<211> 18
<212> DNA
<213> Artificial Sequence

<223> Antisense Oligonucleotide

<400> 85
acacatgaac tccctctg 18

<210> 86
<211> 18
<212> DNA
<213> Artificial Sequence

<223> Antisense Oligonucleotide

<400> 86
atgcatgtta tgagtaac 18

<210> 87
<211> 18
<212> DNA
<213> Artificial Sequence

<223> Antisense Oligonucleotide

<400> 87
tttcaccgca gagatggt 18

<210> 88
<211> 18
<212> DNA
<213> Artificial Sequence

<223> Antisense Oligonucleotide

<400> 88
gaggggtccat actaagcg 18

<210> 89
<211> 18
<212> DNA
<213> Artificial Sequence

<223> Antisense Oligonucleotide

<400> 89
gaggcccgga actccact 18

<210> 90
<211> 18
<212> DNA
<213> Artificial Sequence

<223> Antisense Oligonucleotide

<400> 90
ttccttggtg cctgcgga 18

<210> 91
<211> 18
<212> DNA
<213> Artificial Sequence

<223> Antisense Oligonucleotide

<400> 91
cgtatgagaa ctgcggcg 18

<210> 92
<211> 18
<212> DNA
<213> Artificial Sequence

<223> Antisense Oligonucleotide

<400> 92

aggcccagga gaagtctc 18

<210> 93
<211> 18
<212> DNA
<213> Artificial Sequence

<223> Antisense Oligonucleotide

<400> 93
acgccagagg caggacca 18

<210> 94
<211> 18
<212> DNA
<213> Artificial Sequence

<223> Antisense Oligonucleotide

<400> 94
agtcgagcct cagactcg 18

<210> 95
<211> 18
<212> DNA
<213> Artificial Sequence

<223> Antisense Oligonucleotide

<400> 95
acatacctgc ttccttgt 18

<210> 96
<211> 18
<212> DNA
<213> Artificial Sequence

<223> Antisense Oligonucleotide

<400> 96
ggcgtcggct gatgtcat 18

<210> 97
<211> 18
<212> DNA
<213> Artificial Sequence

<223> Antisense Oligonucleotide

<400> 97
ttcctgcagc agtgccag 18

<210> 98
<211> 18
<212> DNA
<213> Artificial Sequence

<223> Antisense Oligonucleotide

<400> 98
aactttcctc cagccac 18

<210> 99
<211> 18
<212> DNA
<213> Artificial Sequence

<223> Antisense Oligonucleotide

<400> 99
ttctttacag gtattgac 18

<210> 100
<211> 18
<212> DNA
<213> Artificial Sequence

<223> Antisense Oligonucleotide

<400> 100
gcttgaaagc tcttgac 18

<210> 101
<211> 18
<212> DNA
<213> Artificial Sequence

<223> Antisense Oligonucleotide

<400> 101
tctgctgcgt cccaccgg 18

<210> 102
<211> 18
<212> DNA
<213> Artificial Sequence

<223> Antisense Oligonucleotide

<400> 102
gagggagcgg tggatgac 18

<210> 103
<211> 18
<212> DNA
<213> Artificial Sequence

<223> Antisense Oligonucleotide

<400> 103
tcagtcacat ggtcaacc 18

<210> 104
<211> 18

<212> DNA
 <213> Artificial Sequence

 <223> Antisense Oligonucleotide

 <400> 104
 ctctaccat ccactgac 18

 <210> 105
 <211> 18
 <212> DNA
 <213> Artificial Sequence

 <223> Antisense Oligonucleotide

 <400> 105
 ttctgcaatt aatctttt 18

 <210> 106
 <211> 18
 <212> DNA
 <213> Artificial Sequence

 <223> Antisense Oligonucleotide

 <400> 106
 ctgaaaacag actcctct 18

 <210> 107
 <211> 18
 <212> DNA
 <213> Artificial Sequence

 <223> Antisense Oligonucleotide

 <400> 107
 cttcattggc tgcctcct 18

 <210> 108
 <211> 18
 <212> DNA
 <213> Artificial Sequence

 <223> Antisense Oligonucleotide

 <400> 108
 tggatatggtt cttctcag 18

 <210> 109
 <211> 18
 <212> DNA
 <213> Artificial Sequence

 <223> Antisense Oligonucleotide

 <400> 109
 tgctggaagc ctggtatg 18

<210> 110
 <211> 18
 <212> DNA
 <213> Artificial Sequence

 <223> Antisense Oligonucleotide

 <400> 110
 aaccgaggat tatgaagc 18

<210> 111
 <211> 18
 <212> DNA
 <213> Artificial Sequence

 <223> Antisense Oligonucleotide

 <400> 111
 ggcaccggaa ggagatgg 18

<210> 112
 <211> 18
 <212> DNA
 <213> Artificial Sequence

 <223> Antisense Oligonucleotide

 <400> 112
 gacaccagag ggactagc 18

<210> 113
 <211> 18
 <212> DNA
 <213> Artificial Sequence

 <223> Antisense Oligonucleotide

 <400> 113
 cagggagcag ggagtcg 18

<210> 114
 <211> 18
 <212> DNA
 <213> Artificial Sequence

 <223> Antisense Oligonucleotide

 <400> 114
 cagaggtttg cagataca 18

<210> 115
 <211> 18
 <212> DNA
 <213> Artificial Sequence

 <223> Antisense Oligonucleotide

 <400> 115

tgaatggaga agggagaa 18

<210> 116
<211> 18
<212> DNA
<213> Artificial Sequence

<223> Antisense Oligonucleotide

<400> 116
ccagcagatc ccttcctg 18

<210> 117
<211> 18
<212> DNA
<213> Artificial Sequence

<223> Antisense Oligonucleotide

<400> 117
gtaggcagta gtctgact 18

<210> 118
<211> 18
<212> DNA
<213> Artificial Sequence

<223> Antisense Oligonucleotide

<400> 118
ggatgcttgt tcagtcta 18

<210> 119
<211> 18
<212> DNA
<213> Artificial Sequence

<223> Antisense Oligonucleotide

<400> 119
gctccgccca gcacagga 18

<210> 120
<211> 18
<212> DNA
<213> Artificial Sequence

<223> Antisense Oligonucleotide

<400> 120
catccaaaca cagctgct 18

<210> 121
<211> 18
<212> DNA
<213> Artificial Sequence

<223> Antisense Oligonucleotide
 <400> 121
 ttctcatgct gaaatgaa 18
 <210> 122
 <211> 18
 <212> DNA
 <213> Artificial Sequence
 <223> Antisense Oligonucleotide
 <400> 122
 cccggcttca cgtacagc 18
 <210> 123
 <211> 18
 <212> DNA
 <213> Artificial Sequence
 <223> Antisense Oligonucleotide
 <400> 123
 ccagccgctg tccttggt 18
 <210> 124
 <211> 18
 <212> DNA
 <213> Artificial Sequence
 <223> Antisense Oligonucleotide
 <400> 124
 gtctgcagcc cgtatgag 18
 <210> 125
 <211> 18
 <212> DNA
 <213> Artificial Sequence
 <223> Antisense Oligonucleotide
 <400> 125
 ggggtccgccc gcctgggt 18
 <210> 126
 <211> 18
 <212> DNA
 <213> Artificial Sequence
 <223> Antisense Oligonucleotide
 <400> 126
 ttggtaagca gcgagcgc 18
 <210> 127
 <211> 18
 <212> DNA
 <213> Artificial Sequence

<223> Antisense Oligonucleotide

<400> 127
aaggaggtgg aggcccca 18

<210> 128
<211> 18
<212> DNA
<213> Artificial Sequence

<223> Antisense Oligonucleotide

<400> 128
gggagcctta cttgaagg 18

<210> 129
<211> 18
<212> DNA
<213> Artificial Sequence

<223> Antisense Oligonucleotide

<400> 129
gccagaggca ggaccact 18

<210> 130
<211> 18
<212> DNA
<213> Artificial Sequence

<223> Antisense Oligonucleotide

<400> 130
atcctccatc agtcgagc 18

<210> 131
<211> 18
<212> DNA
<213> Artificial Sequence

<223> Antisense Oligonucleotide

<400> 131
tccaaaggtc tcagcaca 18

<210> 132
<211> 18
<212> DNA
<213> Artificial Sequence

<223> Antisense Oligonucleotide

<400> 132
ccttgtgtgg ccacggca 18

<210> 133
<211> 18

<212> DNA
 <213> Artificial Sequence
 <223> Antisense Oligonucleotide

<400> 133
 catcacatac ctgcttcc

18

<210> 134
 <211> 18
 <212> DNA
 <213> Artificial Sequence
 <223> Antisense Oligonucleotide

<400> 134
 gccaggcgctc ggctgatg

18

<210> 135
 <211> 18
 <212> DNA
 <213> Artificial Sequence
 <223> Antisense Oligonucleotide

<400> 135
 caactttcct ccagccca

18

<210> 136
 <211> 18
 <212> DNA
 <213> Artificial Sequence
 <223> Antisense Oligonucleotide

<400> 136
 ccagtagagc cattctct

18

<210> 137
 <211> 18
 <212> DNA
 <213> Artificial Sequence
 <223> Antisense Oligonucleotide

<400> 137
 tgctgcgtcc caccggtg

18

<210> 138
 <211> 18
 <212> DNA
 <213> Artificial Sequence
 <223> Antisense Oligonucleotide

<400> 138
 gctgtggctg cagatttc

18

<210> 139
 <211> 18
 <212> DNA
 <213> Artificial Sequence

 <223> Antisense Oligonucleotide

 <400> 139
 acatggtcaa ccatgagg

18

<210> 140
 <211> 18
 <212> DNA
 <213> Artificial Sequence

 <223> Antisense Oligonucleotide

 <400> 140
 tttaactcct accatcca

18

<210> 141
 <211> 18
 <212> DNA
 <213> Artificial Sequence

 <223> Antisense Oligonucleotide

 <400> 141
 tctgaaaaca gactcctc

18

<210> 142
 <211> 18
 <212> DNA
 <213> Artificial Sequence

 <223> Antisense Oligonucleotide

 <400> 142
 cctgctggaa gcctggta

18

<210> 143
 <211> 18
 <212> DNA
 <213> Artificial Sequence

 <223> Antisense Oligonucleotide

 <400> 143
 atttgaggag atggtgtc

18

<210> 144
 <211> 18
 <212> DNA
 <213> Artificial Sequence

 <223> Antisense Oligonucleotide

 <400> 144

ctttcctttt gttgaggg

18

<210> 145

<211> 18

<212> DNA

<213> Artificial Sequence

<223> Antisense Oligonucleotide

<400> 145

gtatctcaag ggtgatac

18

<210> 146

<211> 18

<212> DNA

<213> Artificial Sequence

<223> Antisense Oligonucleotide

<400> 146

ccccactcaa tgtggttc

18

Docket No.

RTSP-0153

Declaration and Power of Attorney For Patent Application

English Language Declaration

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

Antisense Modulation of SRA Expression

the specification of which

(check one)

☐ is attached hereto.

☒ was filed on 23 March 2000 as United States Application No. or PCT International

Application Number PCT/US00/07634

and was amended on _____

(if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119(a)-(d) or Section 365(b) of any foreign application(s) for patent or inventor's certificate, or Section 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)

Priority Not Claimed

_____ (Number)	_____ (Country)	_____ (Day/Month/Year Filed)	<input type="checkbox"/>
_____ (Number)	_____ (Country)	_____ (Day/Month/Year Filed)	<input type="checkbox"/>
_____ (Number)	_____ (Country)	_____ (Day/Month/Year Filed)	<input type="checkbox"/>

I hereby claim the benefit under 35 U.S.C. Section 119(e) of any United States provisional application(s) listed below:

(Application Serial No.)

(Filing Date)

(Application Serial No.)

(Filing Date)

(Application Serial No.)

(Filing Date)

I hereby claim the benefit under 35 U. S. C. Section 120 of any United States application(s), or Section 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. Section 112, I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, C. F. R., Section 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application:

09/280,409

March 29, 1999

Patented

(Application Serial No.)

(Filing Date)

(Status)
(patented, pending, abandoned)

(Application Serial No.)

(Filing Date)

(Status)
(patented, pending, abandoned)

(Application Serial No.)

(Filing Date)

(Status)
(patented, pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. (list name and registration number)



26259

PATENT TRADEMARK OFFICE

7-

Herb Boswell, Reg. No. 27,311Laurel Spear Bernstein, Reg. No. 37,280Robert S. Andrews, Reg. No. 44,508April Logan, Reg. No. 33,950Neil S. Bartfeld, Reg. No. 32,901Kenneth H. Tarbet, Reg. No. 43,181Donna T. Ward, Reg. No. P48,271

of the firm

ISIS Pharmaceuticals, Inc.-Carlsbad Research Center

2292 Faraday Avenue

Carlsbad, California 92008

Send Correspondence to:

Direct Telephone Calls to: (name and telephone number)

Jane Massey Licata or Kathleen A. Tyrrell - (856) 810-1515

2044720 94926650

Full name of sole or first inventor Lex M. Cowser	
Sole or first inventor's signature <i>[Signature]</i>	Date 8/23/01
Residence San Mateo, California <i>CA</i>	
Citizenship US	
Post Office Address 1299 Parrott Drive	
San Mateo, California 94402	

Full name of second inventor, if any C. Frank Bennett	
Second inventor's signature <i>C. Frank Bennett</i>	Date 9-11-01
Residence Carlsbad, California <i>CA</i>	
Citizenship US	
Post Office Address 1347 Cassins Street	
Carlsbad, California 92008	

Full name of third inventor, if any

Burt W. O'Malley

Third inventor's signature

Date

Residence

Houston, Texas

Citizenship

US

Post Office Address

639 Ramblewood**Houston, Texas 77030**

Full name of fourth inventor, if any

Fourth inventor's signature

Date

Residence

Citizenship

Post Office Address

Full name of fifth inventor, if any

Fifth inventor's signature

Date

Residence

Citizenship

Post Office Address

Full name of sixth inventor, if any

Sixth inventor's signature

Date

Residence

Citizenship

Post Office Address

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
66
67
68
69
70
71
72
73
74
75
76
77
78
79
80
81
82
83
84
85
86
87
88
89
90
91
92
93
94
95
96
97
98
99
100
101
102
103
104
105
106
107
108
109
110
111
112
113
114
115
116
117
118
119
120
121
122
123
124
125
126
127
128
129
130
131
132
133
134
135
136
137
138
139
140
141
142
143
144
145
146
147
148
149
150
151
152
153
154
155
156
157
158
159
160
161
162
163
164
165
166
167
168
169
170
171
172
173
174
175
176
177
178
179
180
181
182
183
184
185
186
187
188
189
190
191
192
193
194
195
196
197
198
199
200
201
202
203
204
205
206
207
208
209
210
211
212
213
214
215
216
217
218
219
220
221
222
223
224
225
226
227
228
229
230
231
232
233
234
235
236
237
238
239
240
241
242
243
244
245
246
247
248
249
250
251
252
253
254
255
256
257
258
259
260
261
262
263
264
265
266
267
268
269
270
271
272
273
274
275
276
277
278
279
280
281
282
283
284
285
286
287
288
289
290
291
292
293
294
295
296
297
298
299
300
301
302
303
304
305
306
307
308
309
310
311
312
313
314
315
316
317
318
319
320
321
322
323
324
325
326
327
328
329
330
331
332
333
334
335
336
337
338
339
340
341
342
343
344
345
346
347
348
349
350
351
352
353
354
355
356
357
358
359
360
361
362
363
364
365
366
367
368
369
370
371
372
373
374
375
376
377
378
379
380
381
382
383
384
385
386
387
388
389
390
391
392
393
394
395
396
397
398
399
400
401
402
403
404
405
406
407
408
409
410
411
412
413
414
415
416
417
418
419
420
421
422
423
424
425
426
427
428
429
430
431
432
433
434
435
436
437
438
439
440
441
442
443
444
445
446
447
448
449
450
451
452
453
454
455
456
457
458
459
460
461
462
463
464
465
466
467
468
469
470
471
472
473
474
475
476
477
478
479
480
481
482
483
484
485
486
487
488
489
490
491
492
493
494
495
496
497
498
499
500
501
502
503
504
505
506
507
508
509
510
511
512
513
514
515
516
517
518
519
520
521
522
523
524
525
526
527
528
529
530
531
532
533
534
535
536
537
538
539
540
541
542
543
544
545
546
547
548
549
550
551
552
553
554
555
556
557
558
559
560
561
562
563
564
565
566
567
568
569
570
571
572
573
574
575
576
577
578
579
580
581
582
583
584
585
586
587
588
589
590
591
592
593
594
595
596
597
598
599
600
601
602
603
604
605
606
607
608
609
610
611
612
613
614
615
616
617
618
619
620
621
622
623
624
625
626
627
628
629
630
631
632
633
634
635
636
637
638
639
640
641
642
643
644
645
646
647
648
649
650
651
652
653
654
655
656
657
658
659
660
661
662
663
664
665
666
667
668
669
670
671
672
673
674
675
676
677
678
679
680
681
682
683
684
685
686
687
688
689
690
691
692
693
694
695
696
697
698
699
700
701
702
703
704
705
706
707
708
709
710
711
712
713
714
715
716
717
718
719
720
721
722
723
724
725
726
727
728
729
730
731
732
733
734
735
736
737
738
739
740
741
742
743
744
745
746
747
748
749
750
751
752
753
754
755
756
757
758
759
760
761
762
763
764
765
766
767
768
769
770
771
772
773
774
775
776
777
778
779
780
781
782
783
784
785
786
787
788
789
790
791
792
793
794
795
796
797
798
799
800
801
802
803
804
805
806
807
808
809
810
811
812
813
814
815
816
817
818
819
820
821
822
823
824
825
826
827
828
829
830
831
832
833
834
835
836
837
838
839
840
841
842
843
844
845
846
847
848
849
850
851
852
853
854
855
856
857
858
859
860
861
862
863
864
865
866
867
868
869
870
871
872
873
874
875
876
877
878
879
880
881
882
883
884
885
886
887
888
889
890
891
892
893
894
895
896
897
898
899
900
901
902
903
904
905
906
907
908
909
910
911
912
913
914
915
916
917
918
919
920
921
922
923
924
925
926
927
928
929
930
931
932
933
934
935
936
937
938
939
940
941
942
943
944
945
946
947
948
949
950
951
952
953
954
955
956
957
958
959
960
961
962
963
964
965
966
967
968
969
970
971
972
973
974
975
976
977
978
979
980
981
982
983
984
985
986
987
988
989
990
991
992
993
994
995
996
997
998
999
1000

Docket No.

RTSP-0153

Declaration and Power of Attorney For Patent Application

English Language Declaration

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

Antisense Modulation of SRA Expression

the specification of which

(check one)

☐ is attached hereto.

☒ was filed on 23 March 2000 as United States Application No. or PCT International

Application Number PCT/US00/07634

and was amended on _____

(if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119(a)-(d) or Section 365(b) of any foreign application(s) for patent or inventor's certificate, or Section 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)

Priority Not Claimed

(Number)

(Country)

(Day/Month/Year Filed)

☐

(Number)

(Country)

(Day/Month/Year Filed)

☐

(Number)

(Country)

(Day/Month/Year Filed)

☐

I hereby claim the benefit under 35 U.S.C. Section 119(e) of any United States provisional application(s) listed below:

(Application Serial No.)

(Filing Date)

(Application Serial No.)

(Filing Date)

(Application Serial No.)

(Filing Date)

I hereby claim the benefit under 35 U. S. C. Section 120 of any United States application(s), or Section 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. Section 112, I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, C. F. R., Section 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application:

09/280,409

March 29, 1999

Patented

(Application Serial No.)

(Filing Date)

(Status)
(patented, pending, abandoned)

(Application Serial No.)

(Filing Date)

(Status)
(patented, pending, abandoned)

(Application Serial No.)

(Filing Date)

(Status)
(patented, pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. *(list name and registration number)*



26259

PATENT TRADEMARK OFFICE

Herb Boswell, Reg. No. 27,311
 Laurel Spear Bernstein, Reg. No. 37,280
 Robert S. Andrews, Reg. No. 44,508
 April Logan, Reg. No. 33,950
 Neil S. Bartfeld, Reg. No. 39,901
 Kenneth H. Tarbet, Reg. No. 43,181
 Donna T. Ward, Reg. No. P48,271
 of the firm
 ISIS Pharmaceuticals, Inc.-Carlsbad Research Center
 2292 Faraday Avenue
 Carlsbad, California 92008

Send Correspondence to:

Direct Telephone Calls to: *(name and telephone number)*

Jane Massey Licata or Kathleen A. Tyrrell - (856) 810-1515

Full name of sole or first inventor Lex M. Cowser	
Sole or first inventor's signature	Date
Residence San Mateo, California	
Citizenship US	
Post Office Address 1299 Parrott Drive	
San Mateo, California 94402	

Full name of second inventor, if any C. Frank Bennett	
Second inventor's signature	Date
Residence Carlsbad, California	
Citizenship US	
Post Office Address 1347 Cassins Street	
Carlsbad, California 92008	

300

Full name of third inventor, if any Bart W. O'Malley jwo 9/7/01	9/7/01
Third inventor's signature <i>Bart W. O'Malley</i>	Date
Residence Houston, Texas TX	
Citizenship US	
Post Office Address 639 Ramblewood	
Houston, Texas 77030	

Full name of fourth inventor, if any	
Fourth inventor's signature	Date
Residence	
Citizenship	
Post Office Address	

Full name of fifth inventor, if any	
Fifth inventor's signature	Date
Residence	
Citizenship	
Post Office Address	

Full name of sixth inventor, if any	
Sixth inventor's signature	Date
Residence	
Citizenship	
Post Office Address	